UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: January 30, 2013

SUBJECT: Joint Science Review with Health Canada Pest Management Regulatory Agency In Support of the Registration of Tea Tree Oil Technical Containing Tea Tree Oil as its Active Ingredient.

Decision Number:	408891 and 408892
DP Number:	396175, 404290 and 407457
EPA File Symbol Number:	86182-E
Chemical Class:	Biochemical
PC Code:	028853
CAS Number:	68647-73-4 and 85085-48-9
Tolerance Exemptions:	Pending- Petition No. 9F7558
MRID Numbers:	48275001, 48275004, 48275007, 48598701-48598704, 48602801, 48878201-48878202, 48995301

FROM: Angela L. Gonzales, Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P) 1/30/13

THROUGH: Felecia A. Fort, Acting Associate Director
Biopesticides & Pollution Prevention Division (7511P) Felecia Fort 1/30/13

TO: Colin Walsh, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

ACTION REQUESTED

In response to the request for additional information discussed in the memorandum from A. L. Gonzales to C. Walsh dated February 28, 2010 and relayed in letters to the applicant dated June 18, 2010 and February 29, 2012, the applicant, Biomor Israel Ltd., has submitted a proposed product label, a Confidential Statement of Formula (CSF) dated October 6, 2010, product chemistry data in MRIDs 48275001, 48275004, and 48995301, mammalian toxicology data in MRIDs 48598701, 48598702, 48598704, 48878201 and 48878202 and nontarget organism toxicology data in MRID 48598703. To support the petition for the exemption from the requirement of a tolerance for residues of the active ingredient, residue data and a screening level dietary risk assessment were submitted in MRIDs 48275007 and 48602801. A request for registration of a proposed end-use product (EP) containing tea tree oil (EPA File Symbol No. 86182-R) has been submitted concurrently and is discussed in a separate memorandum from A. L. Gonzales to C. Walsh dated 1/30/13. EPA is conducting a joint review for these products with Health Canada Pest Management Regulatory Agency (PMRA).

RECOMMENDATIONS AND CONCLUSIONS

1. The product chemistry submission is ACCEPTABLE.
MRID 48275001: ACCEPTABLE
MRID 48275004: ACCEPTABLE
MRID 48995301: SUPPLEMENTAL

a. All product chemistry data requirements have been adequately satisfied.

2. The toxicology submission for the active ingredient is ACCEPTABLE.
MRID 48598701: ACCEPTABLE
MRID 48598702: UPGRADEABLE
MRID 48598704: SUPPLEMENTAL
MRID 48878201: SUPPLEMENTAL
MRID 48878202: ACCEPTABLE

a. All mammalian toxicology data requirements have been adequately satisfied.

3. The nontarget organism toxicology submission is ACCEPTABLE.
MRID 48598703: ACCEPTABLE

a. All nontarget organism toxicology data requirements have been adequately satisfied.

b. Due to the potential for toxicity to freshwater invertebrates, risk assessments will need to be conducted for products containing TTO as an active ingredient for these organisms. Because nontarget organism toxicology data on the proposed EP are available, a risk assessment was conducted for the EP. The assessment is discussed in the memorandum from A.L. Gonzales to C. Walsh dated 1/28/13.

4. The applicant's request for an exemption from the requirement of a tolerance is UNACCEPTABLE.
MRID 48602801: SUPPLEMENTAL
MRID 48275007: ACCEPTABLE

a. Based on the data and information available, the Agency cannot support a justification for the exemption from the requirement of a tolerance for tea tree oil. This conclusion is based on the following:

i. Hazard data indicate toxic endpoints (testicular toxicity in males and developmental toxicity) for tea tree oil. Adverse effects were observed in the 90-day oral toxicity study at dose levels of 60 and 120 mg/kg/day (NOAEL = 30 mg/kg/day). Adverse effects were observed in the developmental study with the surrogate chemical alpha-terpinene (a constituent of tea tree oil) at 60, 125 and 250 mg/kg/day (NOAEL = 30 mg/kg/day). Uncertainties exist regarding the component(s) of tea tree oil responsible for the effects observed in the 90-day oral toxicity study.

ii. Residues may be present on treated commodities. When the proposed end-use product was applied in crop field trials on tomatoes, peppers and cucumbers, no residues of three constituents of tea tree oil that were analyzed were found above the limit of quantification (LOQ). When a product containing 66% TTO was applied to peppers in crop field trials, the residue data indicate that residues of terpinen-4-ol are present after 24 hours. Residues were below the LOQ at 48 hours.

a. Uncertainties are present regarding the potential for residues of the other components of tea tree oil, along with their associated degradates and metabolites when the pesticide is applied to crops in agricultural settings. It is known that the constituents of tea tree oil may be subject to oxidation, photodegradation, etc. once introduced in the environment.

b. Residue data are only available for a small number of food crops (fruiting vegetables and cucurbits)

relative to the number of crops listed on the proposed EP label (tubers, corms, leafy vegetables, tropical and sub-tropical crops, pome fruits, nuts, stone fruits, berries, cereal grains, vine crops and herbs). Moreover, field trials for the crops for which there are residue data are limited in number compared to what is generally required by the Agency.

c. No analysis was conducted for residues of alpha-terpinene in the residue studies submitted to the Agency. Because developmental effects were observed in the study with alpha-terpinene and it is unknown if residues of the substance or its metabolites/degradates would be present on treated crops the Agency cannot make a justification for an exemption from the requirement of a tolerance.

Based on items i-ii above, the Agency cannot support the finding that "any level of exposure to tea tree oil is safe" which is the justification for an exemption from the requirement of a tolerance. Although residues of the three constituents that were tested were not found when the product was used at the proposed EP label rates, residues of one of the constituents were found when tea tree oil was applied at a much higher application rate than that on the proposed EP label. Because the justification for a tolerance exemption is not based on a specific product and must be based on the safety of any level of exposure to the pesticide, the Agency must consider the residue data submitted on the product that was applied at the significantly higher application rate. Additionally, there are uncertainties regarding the potential for degradates and metabolites of tea tree oil to be present on treated crops because only three constituents were analyzed in the residue studies. It is unknown if other residues will be present on treated crops. Because the entity(ies) that is causing the effects observed in the 90-day oral toxicity study is unknown and it is unknown if this particular entity(ies) could be present on treated crops, the Agency cannot make a safety finding to justify an exemption from the requirement of a tolerance. Further, because developmental effects were observed in the developmental toxicity study with alpha-terpinene and it is unknown if residues of the substance or its metabolites/degradates would be present on treated crops the Agency cannot make a justification for an exemption from the requirement of a tolerance.

b. To support a tolerance for residues of tea tree oil, the following data and information are required to be addressed:

i. Nature of the residue studies (OCSPP Guideline 860.1300). A characterization of metabolic, degradation, etc. pathways for the major constituents of tea tree oil is required to ascertain if residues (other than the residues of tea tree oil that have already been identified) may be present on treated commodities after application of the pesticide. This information is required due to the uncertainties regarding the identity of the toxic entity(ies) present in TTO; it is unknown if this entity(ies) could be present on treated crops. If the analysis/characterization reveals residues other than what have already been identified, magnitude of the residue (OCSPP Guidelines 860.1480, 860.1500 and/or 860.1520) studies and additional analytical method (OCSPP Guideline 860.1340) data may be required.

c. The applicant must provide information regarding the correlation between the application rates in the residue studies in MRID 48275007 and the application rates on the proposed EP label.

RISK ASSESSMENT AND STUDY SUMMARIES

Note: The proposed EP, Timorex Gold (EPA File Symbol No. 86182-R) which is referenced below is also identified in the submitted materials as BM 608. The registrant has stated that these products are synonymous; BM 608 is Timorex Gold.

I. Active Ingredient Characterization

Refer to the memorandum from A. L. Gonzales to C. Walsh dated 02/28/10 for this information.

A. Product Chemistry (MRIDs 48275001, 48275004, and 48995301)

All product chemistry data requirements have been adequately satisfied. Viscosity data were submitted and are summarized in Table 1 below. All CSF deficiencies have been resolved. Refer to the Data Evaluation Record (DER) for more information. Refer to the memorandum from A. L. Gonzales to C. Walsh dated 02/28/10 for information regarding the original product chemistry submission.

TABLE 1. Physical and Chemical Properties of Tea Tree Oil Technical (40 CFR § 158.2030)

OPPTS Guideline No.	Property	Description of Result	MRID
830.7100	Viscosity	2.47 cSt at 25°C	48275001

II. Human Health Assessment

A. Toxicology (MRIDs 48598701, 48598702, 48598704, 48878201 and 48878202)

Adequate mammalian toxicology data and information have been submitted to satisfy the Tier 1 toxicology data requirements. The data presented in Table 2 below are a summary of the toxicity data and information submitted to support the proposed MP, Tea Tree Oil Technical.

Inert ingredient information may be entitled to confidential treatment

Table 2. Mammalian Toxicology Data Requirements for Tea Tree Oil Technical (40 CFR § 158.2050)			
Study/OPPTS Guideline No.	**Results**	**Toxicity Category/Description**	**MRID**
Acute oral toxicity (rat) (870.1100)	LD_{50} = 1752 mg/kg (1752-2450 mg/kg)	III	47730404
Acute dermal toxicity (rabbits) (870.1200)	LD_{50} > 2000 mg/kg	III	47730404
Acute inhalation toxicity (870.1300)	LC_{50} = 3.64 mg/L	IV	48598701 48878201
Primary eye irritation (870.2400)	Waived: MP is corrosive to skin. A chorioallantioic membrane vascular assay (chicken egg) was submitted, but is unacceptable as EPA has not established this assay to be appropriate to fulfill the primary eye irritation data requirement.	I	47730405
Primary dermal irritation (rabbit) (870.2500)	Corrosive. Well-defined to severe erythema and barely perceptible to slight edema were noted on the intact sites of 6/6 rabbits at the 24- and 72-hour evaluations. Well-defined to severe erythema and barely perceptible to moderate edema were noted on the abraded sites of 6/6 rabbits at the 24 and 72 hour evaluations. Three animals had erosion on abraded sites at the 72-hour evaluation. The primary irritation index (PII) was 5.0.	I	47730404
Dermal sensitization (guinea pig) (870.2600)	Waived: MP is corrosive to skin. A skin sensitization study was submitted. After intradermal and epidermal inductions, the test and control animals showed no signs of reactivity 24 hours after challenge. However, study classification is unacceptable due to the lack of positive control data.		47730404
Hypersensitivity incidents (885.3400)	Any incidents must be reported		
90-Day oral toxicity (870.3100)	LOAEL = 60 mg/kg/day based on testicular toxicity in males and increased liver to body weight ratios in females NOAEL = 30 mg/kg/day for males and females		48598702 48878202
90-Day dermal toxicity (870.3250)	Waived: prolonged dermal exposure not anticipated. TGAI is corrosive to skin and adequate PPE is required on the label. Undiluted TTO caused severe irritation to the skin on the first day of a non-guideline 30-day dermal irritation study on rabbits. For the remainder of the study, a 25% solution in [redacted] was applied to the skin which did not result in visible irritation; however, non-specific microscopic dermatitis consistent with irritation was observed.		47730411 47730404
90-Day inhalation toxicity (870.3465)	The data from the 90-data oral toxicity study has been bridged to satisfy this data requirement. A route to route extrapolation will be employed using a 100% absorption assumption.		48598704 48878201
Mutagenicity (870.5100, 5300 and 5375)	Not mutagenic in *Salmonella* reverse mutation assay at doses below 50 µg. Negative in *in vivo* mouse bone marrow micronucleus assay.		47730404 47730407 47730409

Table 2. Mammalian Toxicology Data Requirements for Tea Tree Oil Technical (40 CFR § 158.2050)			
Study/OPPTS Guideline No.	**Results**	**Toxicity Category/Description**	**MRID**
Developmental toxicity (870.3700)	Data on an acceptable surrogate chemical (alpha-terpinene) were cited to satisfy the data requirement. Maternal LOAEL = 125 mg/kg/day based on dose- and treatment- related reduced body weight gain. Maternal NOAEL = 60 mg/kg/day. Developmental LOAEL = 60 mg/kg/day based on abnormal ossification of bones and minor skeletal abnormalities in fetuses Developmental NOAEL = 30 mg/kg/day		48598704 48878201

Refer to the DERs for more information.

1. Acute Toxicity

An acute inhalation study conducted on the active ingredient was submitted in MRID 48598701. Additional information regarding the study was submitted in MRID 48878201. Based on the available data, TTO is placed into Toxicity Category IV for acute inhalation toxicity. Refer to the DER for more information for MRID 48598701. A DER was not created for MRID 48878201. In this MRID, adequate justification was provided regarding the reason why only some of the major components of TTO were analyzed in the acute inhalation study rather than analyzing all of the major components. Other information regarding analysis of TTO was also submitted in the MRID; however, this information was already provided in MRID 48598701.

For a summary of all other acute toxicity data, refer to the memorandum from A. L. Gonzales to C. Walsh dated 2/28/10.

2. Subchronic Toxicity

90-Day Oral

A 90-day oral toxicity study was conducted on TTO and submitted in MRID 48598702. Adequate supportive information regarding homogeneity testing of the test substance was submitted in MRID 48878202. Tea tree oil was administered to Wistar rats by gavage at dosages of 0 (peanut oil), 30, 60 or 120 mg/kg/day. An additional 20 rats were included in the high dose group, and an additional 10 rats were included in the control group. To determine the reversibility of any effects observed, 10 rats from the high dose group and 5 rats from the control group were sacrificed 14 days and 28 days after completion of the 90-day dosing period. No mortality was observed in male rats. Two moribund females in the high dose group were sacrificed during the treatment period. At all dose levels salivation was observed which persisted for about 30 minutes after dosing. Salivation incidences were dose-related. No treatment-related effects on body weight and body weight gain were observed. Toxicologically relevant hematological, clinical chemistry or urinalysis findings were not noted at any dose level. In the high dose group, some motor activity measurements were statistically ($p \leq 0.05$) affected in both sexes; however, the toxicological significance of these observations is unclear as there was no correlation with abnormal functional observations. Statistically significant increases in liver to body weight ratios at the mid- and high dose levels (+11% and +9%, respectively) were noted in females, and a significant increase in ovary to body weight ratio was noted in the 28-day high dose recovery group (+31%). There was no microscopic correlation for the organ weight findings in females. Flaccid testes, some with a small appearance, were observed in high-dose males (4/10 at the end of treatment; 2/10 after the 14-day recovery period; 8/10 after the

28-day recovery period). Absolute organ weight and organ-to-body-weight ratios were significantly reduced in the testes and epididymides of males in the high-dose 28-day recovery group. These findings were microscopically associated with degenerative changes in the seminiferous tubules (loss of germ cells, formation of multinucleated germ cells, presence of cell debris in tubular lumen, atrophic appearance, and sertoli cell vacuolation). The testicular effects occurred at the same or higher rate of incidence with an overall greater degree of severity in the high dose animals in the 14-day and 28-day recovery groups. Sperm granulomas and cellular debris in the lumen (inflammation involving epididymal lumen and interstitium with the presence of large zones of spermatozoa surrounded by a zone of macrophages, giant cells and fibroblasts) were observed in the epididymides of high dose males at the end of the 90-day treatment period. The incidence of sperm granulomas peaked in the 14-day recovery group, but the incidence and severity of cellular debris in the epididymal lumen increased in the 14- and 28-day recovery groups. Minimal cell debris was observed in the lumen of the epididymides in 1/10 male rats dosed at 60 mg/kg/day. No treatment-related histological changes were observed in males dosed at 30 mg/kg/day. Statistically significant treatment-related effects on sperm motility, sperm morphology, and epididymal sperm counts were observed at the high dose level at the end of the treatment period and in the 14- and 28-day recovery groups. Sperm counts were significantly lower relative to controls in the mid-dose group, and there was a significant decrease in sperm motility and an insignificant increase in abnormal sperm at this dose level. Based on these data, the lowest-observed-adverse-effect level (LOAEL) of tea tree oil in rats was 60 mg/kg/day in males based on testicular toxicity in males and increased liver to body weight ratios in females. The no-observed-adverse-effect level (NOAEL) is 30 mg/kg/day for males and females.

90-Day Dermal

A 90-day dermal study was not submitted and is not required. Exposure via the dermal route is not anticipated due to the following: 1) prolonged dermal exposure is not expected because the product is not purposely applied to the skin and handlers/applicators are required to wear appropriate PPE; 2) a 4-hour restricted-entry interval (REI) requirement has been included on the proposed EP label which will further mitigate exposure; and 3) TTO has a long history of use in a variety of dermally-applied products, including shampoo, deodorants, lotions, and antifungal treatments. In a non-guideline 30-day dermal irritation study on rabbits, undiluted TTO caused severe irritation to the skin on the first day of the study. For the remainder of the study, a 25% solution in [redacted] was applied to the skin which did not result in visible irritation; however, non-specific microscopic dermatitis consistent with irritation was observed. Refer to the DER for more information.

90-Day Inhalation

A 90-day inhalation toxicity study was not submitted for TTO. In lieu of a study, the applicant cited the data from the 90-day oral toxicity study (MRID 48598702; see summary above). Due to the use pattern of the active ingredient as a spray and the volatility of the constituents of TTO, exposure to handlers/applicators is possible. There are no PPE requirements regarding mitigation of inhalation exposure on the proposed EP label. A route to route extrapolation using the default assumption that 100% of the TTO is absorbed into the body has been employed. The Agency is confident that this approach is protective of potential repeat exposure inhalation toxicity for the following reasons: 1) tea tree oil is classified into Toxicity Category IV for acute inhalation toxicity; it is classified into Toxicity Category III for acute oral toxicity, 2) humans are already exposed to the constituents of tea tree oil as the oil is used in a variety of dermally-applied cosmetic and pharmaceutical products. Additionally, five of the main components of TTO (terpinen-4-ol, γ-terpinine, α-terpinine, p-cymene and 1,8-cineole) are all approved for use by the Food and Drug Administration (FDA) as direct food additives under 21 CFR 172.515, and 3) in a occupational risk assessment conducted for applicators and handlers which

may be exposed via inhalation to tea tree oil, margins of exposure (MOEs) were derived and were significantly higher than the Agency's LOC of 100 (MOEs ranged from 4,900 to 150,000). Because the MOEs were significantly higher than the LOC, the Agency believes that an adequate margin of safety has been achieved to account for any potential difference in toxicity from oral versus inhalation exposure.

3. Developmental Toxicity and Mutagenicity

A developmental toxicity study conducted on TTO is not available. A developmental toxicity study conducted on terpinen-4-ol, the residue identified in the residue studies is also not available. Because terpinen-4-ol has been the only residue identified thus far, the Agency requested developmental toxicity data for TTO or terpinen-4-ol. In lieu of a study on either substance, the applicant cited developmental toxicity data on a surrogate chemical, alpha-terpinene (α-terpinene), in MRID 48598704. Adequate rationale to bridge the data on α-terpinene to TTO or terpinen-4-ol was provided in MRID 48878201.

Development toxicity data for α-terpinene are sufficient to satisfy the developmental toxicity data requirement. Alpha-terpinene is an acceptable surrogate chemical due to its presence as a component in TTO, its structural similarities to terpinen-4-ol and its metabolic pathway similarities to terpinen-4-ol. Tea tree oil is comprised of terpene hydrocarbons, primarily monoterpenes, sesquiterpenes and associated alcohols. Alpha-terpinene, a monoterpene hydrocarbon, is a constituent in tea tree oil and is present in a range of 5-13% in the source of the oil used as a pesticide. Terpinen-4-ol is an associated alcohol and is present as a constituent of tea tree oil in a range of 30-48%. Data submitted in MRID 48878201 regarding synthesis pathways for the two chemicals demonstrate that they can be derived from the same parent compound. Metabolism data and information submitted in MRID 48878201 indicate that α-terpinene and terpinen-4-ol are metabolized in a similar manner in humans. According to papers by the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives (JECFA, 2000 and 2006) and Haigou and Miyazawa (2012), both chemicals are metabolized by one of two possible routes, either: 1) allylic oxidation, epoxidation and hydrolysis to form diols or, 2) conjugation with glucuronic acid and excretion in the urine. The data and information discussed above suggest that exposure to either α-terpinene or terpinen-4-ol would result in similar metabolic pathways and subsequent exposure to similar metabolites. Based on this reasoning, it can be inferred that toxicological effects for α-terpinene could be representative of toxicological effects for terpinen-4-ol.

A developmental toxicity study was cited from the open scientific literature in MRID 48598704 in which α-terpinene (89% purity) was administered to groups of female rats at dose levels of 0, 30, 60, 125 or 250 mg/kg bw/day. Reduced body weight gain in the female rats was observed at the two highest doses. The pregnancy rate was significantly decreased at the highest dose. Reduced fetal body weight and increased fetal kidney weights were observed at the highest dose tested. Minor skeletal abnormalities and delayed bone ossification were observed in fetuses in the 60 mg/kg/day dose group. The maternal LOAEL was 125 mg/kg/day based on decreased body weight gain; the maternal NOAEL was 60 mg/kg/day. The developmental LOAEL was 60 mg/kg/day based on minor skeletal abnormalities and abnormal ossification of bones; the developmental NOAEL was 30 mg/kg/day. Refer to the DER for more information.

A search of the National Library of Medicine's Toxicology Data Network (TOXNET) on January 18, 2013 for developmental endpoints for the 15 major constituents of TTO was conducted and endpoints were identified for alpha-terpinene (the study which is summarized above), alpha-pinene and limonene. The doses at which endpoints were identified were all higher than the NOAEL for the developmental toxicity study on alpha-terpinene. This finding suggests that the NOAEL selected for TTO (the NOAEL for alpha-terpinene) does not under-represent the potential developmental toxicity of TTO.

For a summary of the mutagenicity data, refer to the memorandum from A. L. Gonzales to C. Walsh dated 2/28/10.

Note: A DER was not created for MRID 48878201.

4. Effects on the Endocrine System

Refer to the memorandum from A. L. Gonzales to C. Walsh dated 2/28/10.

B. Dose Response Assessment

The NOAEL of 30 mg/kg bw/day has been selected as the point of departure (POD) for the hazard and risk assessment. The NOAEL is based on toxic endpoints observed in the 90-day oral toxicity study on TTO and the developmental endpoints observed in the developmental toxicity study on α-terpinene.

C. Food Quality Protection Act (FQPA) Considerations

1. Dietary Exposure and Risk Characterization

At this time, based on the data available to the Agency, an exemption from the requirement of a tolerance for tea tree oil cannot be justified. This conclusion is based on the following: 1) residue studies indicate that dietary exposure to residues of at least one of the constituents of tea tree oil may occur when tea tree oil is used as a pesticide on food commodities; 2) toxicological endpoints (discussed above) have been identified in both the 90-day oral and developmental toxicity studies; and 3) uncertainties exist regarding the identity of potential residues that may be found on treated commodities. For these reasons, the Agency cannot conclude that, "any level of exposure of residues of tea tree oil is safe", which is the justification for an exemption from the requirement of a tolerance.

The residue study (MRID 47730522) which indicated residues of terpinen-4-ol were present at 24-hours post application of a product containing 66% TTO is discussed in the memorandum from A. L. Gonzales to C. Walsh dated 2/28/10. Refer to the DER for this MRID for additional information. Because of the uncertainty regarding the application rates used in the studies, the applicant submitted information to clarify the application rates which indicates that the application rates were significantly different than the Agency's original supposition. The actual application rates in the studies in MRID 47730522 ranged from 1,190 g a.i./hectare to 13,200 g a.i./hectare. The maximum label application rate is 1,788 g a.i./hectare.

An additional residue study was submitted in MRID 48275007. In the study, the proposed end-use product (EPA File Symbol No. 86182-R) was applied indoors on tomatoes, sweet peppers and cucumbers at 0.5 L/hl (119 g a.i./hl) and at 1.0 L/hl (238 g a.i./hl). The relationship between these application rates and the application rates on the proposed product label is uncertain and was not clearly defined in the MRID. Six trials were conducted; one on tomatoes in Italy, one on peppers in Greece and four on cucumbers in France, Germany, the Netherlands and the United Kingdom (UK). At all sites, samples were obtained by hand for analysis immediately after the pesticide had dried and at 6 hours post-application. In France and Germany, samples were also collected at 12 hours after application and in the UK, samples were collected at 24 and 48 hours post-application. Samples were analyzed for residues of 3 of the major constituents in tea tree oil, terpinen-4-ol, gamma-terpinene and 1,8-cineole. No residues of any of these three constituents above the limit of

quantification (0.05 mg/kg) were identified at any site for all crops for both application rates. Refer to the DER for more information.

Methyl Eugenol

Data on the methyl eugenol content in the source of TTO used as a pesticide were requested by PMRA. These data were requested to support the dietary risk assessment. Because EPA and PMRA are conducting a joint review, these data were also submitted to EPA. The following is a summary and review of the submitted data.

Methyl eugenol (ME) is a constituent of tea tree oil, the identity and content of which is not specified in the ISO Standard 4730 for the oil. According to the National Toxicology Program (NTP), methyl eugenol is reasonably anticipated to be a human carcinogen based on evidence of carcinogenicity in experimental animals (HSDB, 2013). Although the substance is reasonably anticipated to be a carcinogen, the amount in TTO is considered to be insignificant and not of concern. Methyl eugenol is a naturally occurring substance found in a variety of foods, including spices, herbs and fruit. It is also a component of essential oils, many of which are commonly used (e.g.: basil, rose and clove oils). (Southwell, 2011) The Food and Drug Administration (FDA) has approved its use as a food additive under 21 CFR 172. 515. The EPA allows ME's use as a fragrance (with limitations) in pesticide products. In a study from the open scientific literature, the content of ME in terpinen-4-ol chemotype TTO (the type that will be used as a pesticide) was quantified and ranged from less than 0.01% to 0.06% in TTO. One-hundred twenty-eight samples of commercial TTO were analyzed; ME content ranged from 0-554 ppm, with a mean of 206 ppm. (Southwell, 2011) The authors of the study concluded that the risk of ME exposure in tea tree oil when used as recommended is negligible. A comparison was made between the amount of a "normal dose" of methyl eugenol from dermal application of tea tree oil to the amounts of the chemical required to achieve carcinogenicity and the authors determined that tens of thousands of doses of tea tree oil would be required to reach a harmful dose of methyl eugenol. The applicant submitted information on the content of methyl eugenol in their source of tea tree oil in MRID 48995301. An analysis of five batches of TTO was conducted for the content of ME. The average concentration was 39.4 ppm with a range of 26.5 to 49.5 ppm. These concentrations are significantly lower than the concentrations found in the Southwell study. Potential human exposure to ME is anticipated to be further reduced due to the substance's relatively rapid degradation in the environment. Environmental fate processes include volatilization or biodegradation. (HSDB, 2013) The data and information presented above indicate that potential exposure (if any) to ME in the source of TTO used as a pesticide is not likely to result in unreasonable adverse effects in humans.

2. Drinking Water Exposure and Risk Characterization

The assessment will be completed once residue data requirements have been adequately fulfilled.

3. Acute and Chronic Dietary Risks for Sensitive Subpopulations Particularly Infants and Children

The assessment will be completed once residue data requirements have been adequately fulfilled.

D. Occupational, Residential, School and Day Care Exposure and Risk Characterization

1. Occupational Exposure and Risk Characterization

Dermal exposure to mixers/loaders and applicators is not anticipated as adequate personal protective equipment (PPE) requirements are on the proposed EP label. Additionally, the active ingredient degrades rapidly in the environment which further mitigates dermal exposure. Due to the potential for inhalation exposure to mixers/loaders and applicators to tea tree oil (the EP is applied as a spray, fog or drench) and the effects observed in the 90-day oral and developmental studies, an occupational exposure and risk assessment was conducted. The results of the risk assessment indicate that risk from inhalation exposure to tea tree oil is negligible for mixers/loaders and applicators when the pesticide product (Timorex Gold [EPA File Symbol No. 86182-R]) is used according to label instructions. A summary of the assessment is provided below.

The assessment was conducted using a POD of 30 mg/kg bw/day (the NOAEL from the 90-day oral and developmental toxicity studies) and the occupational handler exposure calculation spreadsheet developed in OPP's Health Effects Division (HED). Because 90-day inhalation toxicity data are not available, the Agency has bridged the data from the 90-day oral toxicity study and assumed 100% absorption via inhalation. The standard body weight for females (69 kg) was used for all exposure scenarios in the assessment due to the incidence of developmental effects. Based on label instructions, exposure scenarios consisted of the following application methods: ground boom, airblast, aerial and mechanically-pressured handgun. The application rate used for the exposure assessment was the maximum application rate on the label (1.6 lb TTO/acre). Potential daily exposures for occupational handlers (mixer/loaders and applicators) were calculated in the spreadsheet using the following formulas:

Daily exposure to the pesticide:

*Daily Exposure (mg ai /day) = UE (µg ai / lb ai) * AR (lb ai /A) * AT (A /day) * 1E-3 mg/µg*

Daily Exposure = amount of the active ingredient that is available for inhalation absorption,
UE = Unit Exposure (generic values derived using measurements of exposure data in the field)
AR = maximum application rate according to proposed label
AT = daily acres treated.

The average daily dose (daily exposure adjusted for absorption and body weight) was calculated using the following formula:

$$\textit{Average Daily Dose (mg ai/kg/day)} = \frac{\textit{[Daily Exposure (mg ai/day) * Absorption (\%)]}}{\textit{Body Weight (kg)}}$$

Average Daily Dose (ADD) = absorbed dose received from exposure to the active ingredient in a given scenario
Daily Exposure = amount of the active ingredient that is available for inhalation absorption
Absorption Factor = a measure of the amount of chemical that crosses a biological boundary such as the skin and lungs (%)
Body Weight = body weight determined to represent the population of interest in a risk assessment

Risk for each application handler scenario was calculated using a Margin of Exposure (MOE), which is a ratio of the toxicological endpoint to the daily dose of concern. The daily inhalation dose received by occupational

handlers was compared to the POD (i.e.: NOAEL) to assess the risk to occupational handlers. All MOE values were calculated using the following formula:

$$MOE = \frac{POD \text{ (i.e.: NOAEL in mg/kg/day)}}{ADD \text{ (mg/kg/day)}}$$

All MOEs calculated for mixers/loaders and applicators using no inhalation PPE were significantly greater than the Agency's Level of Concern (LOC) of 100 (10X for intraspecies variation and 10x for interspecies variation). Margins of exposure greater than 100 do not exceed the Agency's LOC; therefore, risks are not considered to be of concern. Based on the results of the occupational risk assessment, unreasonable adverse effects to handlers are not anticipated when the pesticide product is used according to label instructions. A summary of the exposure scenarios and MOEs is provided in Table 3 below.

TABLE 3. Occupational Risk Assessment Summaries for Tea Tree Oil (Inhalation Route of Exposure)

Worker Activity	Application Equipment/Application Type	Application Type	MOE
Mixer/Loader	Aerial/Broadcast	Orchard	17,000
Mixer/Loader	Aerial/Broadcast	Field crop, typical	17,000
Mixer/Loader	Aerial/Broadcast	Field crop, high-acreage	4,900
Mixer/Loader	Airblast/Broadcast	Orchard	150,000
Mixer/Loader	Groundboom/Broadcast	Field crop, typical	74,000
Mixer/Loader	Groundboom/Broadcast	Field crop, high-acreage	29,000
Applicator	Aerial/Broadcast	Orchard	Exposure not anticipated
Applicator	Aerial/Broadcast	Field crop, typical	Exposure not anticipated
Applicator	Aerial/Broadcast	Field crop, high-acreage	Exposure not anticipated
Applicator	Airblast/Broadcast	Orchard	6,900
Applicator	Groundboom/Broadcast	Field crop, typical	48,000
Applicator	Groundboom/Broadcast	Field crop, high-acreage	19,000
Flagger	Aerial/Broadcast	Orchard	11,000
Flagger	Aerial/Broadcast	Field crop, typical	11,000
Flagger	Aerial/Broadcast	Field crop, high-acreage	11,000

Significant post-application exposure to tea tree oil is not anticipated as there is a 4-hour Restricted Entry Interval (REI) on the label and the active ingredient is expected to rapidly degrade in the environment.

2. Residential, School and Day Care Exposure and Risk Characterization

Significant exposure to TTO is not anticipated in residential, school, and day care areas, as the product containing this active ingredient is intended for use on horticultural and agricultural crops.

E. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

The assessment will be completed once residue data requirements have been adequately fulfilled.

F. Cumulative Effects

The assessment will be completed once residue data requirements have been adequately fulfilled.

G. Risk Characterization

The risk characterization will be completed once residue data requirements have been adequately fulfilled.

III. Environmental Assessment

A. Nontarget Organism Toxicology (MRID 48598703)

Adequate nontarget organism toxicology data and information have been submitted to satisfy the nontarget organism toxicity data requirements for the TGAI. Data on the proposed EP, Timorex Gold (EPA File Symbol No. 86182-R) were also submitted and are summarized in the memorandum from A. L. Gonzales to C. Walsh dated 2/28/10. The data presented in Table 4 below are a summary of the nontarget organism toxicity data and information submitted to support the proposed MP, Tea Tree Oil Technical.

TABLE 4. Non-Target Organism Toxicity Requirements for Tea Tree Oil Technical (40 CFR § 158.2060)

Study/OPPTS Guideline No. /MRID No.	Results	Toxicity Category/Description	MRID
Avian acute oral toxicity *Coturnix coturnix japonica* (850.2100)	Adequate information to support data requirement: significant exposure to birds is not expected due to volatilization and rapid degradation of the active ingredient in the environment. Additionally, components of the TGAI are already found in a multitude of plants.	Significant exposure not expected	47730411
Avian dietary toxicity *Colinus virginianus* (850.2200)	Adequate information to support data requirement: significant exposure to birds is not expected due to volatilization and rapid degradation of the active ingredient in the environment. Additionally, components of the TGAI are already found in a multitude of plants.	Significant exposure not expected	47730411
Aquatic invertebrate acute toxicity (*Daphnia magna*) (850.1010)	48-hour EC_{50} = 0.591 mg/L (95% CL: 0.499-0.700 mg/L)	Highly toxic	48598703
Freshwater fish LC_{50} (*Brachydanio rerio*) (850.1075)	LC_{50} > 100 mg/L	Practically non-toxic	47730408
Non-target plant studies (850.4000-4800, as applicable)	Adequate information to support data requirement: using the proposed EP in efficacy trials on squash, potatoes, apples, no phytotoxicity was observed. Minor phytotoxicity was observed in one South African grape variety (brown spots) in one trial; however, in nine other trials in grapes no phytotoxicity was observed. Minor phytotoxicity was observed in one trial in tomatoes and cucumbers (small necrotic spots); however, no phytoxicity was observed in eleven other trials in tomatoes. The components of TTO are volatile and are also already found in a multitude of plants.	Not phytotoxic	47730521

Non-target insect testing (880.4350)	*Apis millifera*: oral LD_{50} > 98.5 µg a.i./bee; contact LD_{50} > 331 µg a.i./bee *Bombus terrestris:* oral LD_{50} > 105.49 µg a.i./bee; contact LD_{50} > 100 µg a.i./bee *Aphidius rhopalosiphi*: ER_{50} and LR_{50} >1.08 L **EP**/ha *Typhlodromus pyri*: ER_{50} = 1.78 L EP/ha (95% c.i., 1.54-2.09 L **EP**/ha); 7d LR_{50} >4.32 L EP/ha	Practically non-toxic	47730520[1] 47730519[2] 47730518[3] 47730517[3]

[1] Study conducted on the proposed EP, Timorex Gold (EPA File Symbol No. 86182-R) at target doses of 100 µg a.i./bee (oral) and 18, 32, 56, 100, 180, and 320 µg a.i./bee (contact)

[2] Study conducted on the proposed EP, Timorex Gold (EPA File Symbol No. 86182-R) at a target dose of 100 µg a.i./bee (nominally equivalent to 423.73 µg EP/bee)

[3] Supplemental data. Values reported are based on the proposed EP Timorex Gold (EPA File Symbol No. 86182-R) and have not been corrected for active ingredient concentration.

Refer to the DERs for more information.

B. Environmental Fate and Groundwater Data

Tea tree oil is expected to degrade rapidly in the environment. Up to 90% of TTO has been shown to volatilize within 24 hours after application and residue studies indicate the lack of detectable residues of three of the major constituents of TTO at 48 hours post-application (Chadwick, 2008; Gatenio and Ofer, 2007; MRID 47730409). In MRID 48275004, a study was submitted in which the biodegradation of TTO was determined in a 28-day ready biodegradability assay (OECD Guideline 310 and EN ISO 14593) by monitoring the release of carbon dioxide with a non-adapted activated sewage sludge. Based on the results of the assay, biodegradation of TTO was 10% after 2 days, 60% after 5 days, 87% by day 7 and 106% at study termination.

C. Ecological Exposure and Risk Characterization

Significant exposure to birds is not expected because the active ingredient is volatile and degrades rapidly in the environment. Additionally, birds are already exposed to the components of TTO, as they are present in a multitude of plants. Although exposure to fish, insects and plants is anticipated from use of the product through direct application, accidental application, drift or run-off, results of toxicity testing on pure TTO indicate that TTO is practically nontoxic to these organisms. The proposed EP which contains 23.8% TTO will also be diluted with water prior to application, which will further reduce exposure to these organisms. The aquatic invertebrate toxicity study submitted indicates that TTO is highly toxic to these organisms. Because of the potential for toxicity to freshwater invertebrates, risk assessments for these organisms will need to be conducted for products containing TTO as an active ingredient. Since nontarget organism toxicology data on the proposed EP are available, a risk assessment was conducted for the EP. The assessment is discussed in the memorandum from A. L. Gonzales to C. Walsh dated 1/28/13.

D. Endangered Species Assessment

The Agency has not conducted a risk assessment that supports a complete endangered species determination. The ecological risk assessment planned during registration review will allow the Agency to determine whether tea tree oil's use has "no effect" or "may effect" federally listed threatened or endangered species (listed species) or their designated critical habitats. When an assessment concludes that a pesticide's use "may affect" a listed species or its designated critical habitat, the Agency will consult with the U.S. Fish and Wildlife Service and/or National Marine Fisheries Services (the Services) as appropriate.

REFERENCES

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Southwell I.A., M.F. Russell and N.W. Davies. (2011). Detecting traces of methyl eugenol in essential oils: tea tree oil, a case study. Flavour Fragr. J. 26: 336-340.

cc: Angela L. Gonzales, Felecia Fort, Colin Walsh, BPPD Science Review File, IHAD/ARS
A. L. Gonzales, FT, PY-S: 1/30/13

DATA EVALUATION RECORD

BM608
[TIMOREX GOLD - TEA TREE OIL]

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT [OCSPP 870.2500]
MRID 48275006

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Donna L. Fefee, AE
Date: JAN 04 2012

Secondary Reviewers:
Thomas C. Marshall, Ph.D., D.A.B.T.

Signature: Thomas C. Marshall. AE
Date: JAN 04 2012

Robert H. Ross, M.S., Program Manager

Signature:
Date: JAN 04 2012

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg
Date: JAN 04 2012

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DATA EVALUATION RECORD

BM608
[TIMOREX GOLD - TEA TREE OIL]

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT [OCSPP 870.2500]
MRID 48275006

Prepared for
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9724 Kingston Pike, Suite 602
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Date: _____

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: _____
Date: _____

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DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales 1/26/13 /s/**
STUDY TYPE:	Primary Dermal Irritation - Rabbits (OCSPP 870.2500)
MRID NO:	48275006
DP BARCODE NO:	396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	BM 608 (Batch # 6345) (Timorex Gold)
PROJECT NO:	29832 (Laboratory Study Number)
SPONSOR:	Stockton Crop Protection AG, 112 Baarer Str. CH-6302, Zug, Switzerland
TESTING FACILITY:	Eurofins PSL, 2394 US Highway, 130 Suite E, Dayton, New Jersey 08810.
TITLE OF REPORT:	BM 608: Primary Skin Irritation Study in Rabbits
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	September 2, 2010
GOOD LABORATORY PRACTICE:	Study meets requirements of 40 CFR Part 160; specific information related to the characterization of the test material was the responsibility of the sponsor.
CONCLUSION:	The Primary Irritation Index was 4.00, and BM 608 (Tea Tree Oil Technical) was a moderate irritant (per US EPA 1988).
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

I. STUDY DESIGN:

1. **Test Material:** BM 608; nominal composition: 23.14% Tea Tree Oil (a.i.) and 76.86% other ingredients; Batch No.: 6345; PSL Reference No.: 100426-8H; yellow-light brown liquid; pH: 9.15; soluble in water; expiration date: December 22, 2011; stored at room temperature and expected to be stable for the duration of testing.

2. **Test Animals:** Three young adult female New Zealand albino rabbits were received from Robinson Services, Inc. (Clemmons, N.C.), identified using stainless-steel ear tags (#3501-#3503), and acclimated for 6 days. Housing was individual in suspended stainless-steel cages with mesh floors. The animals were fed pelleted Purina Rabbit Chow #5326 and supplied with *ad libitum* filtered tap water. The environmental conditions of the animal room were as follows: temperature, 19-22° C; relative humidity, 49-75%;[a] air changes, not reported; and photoperiod, 12 hour light/dark cycle. The exact ages and body weights of the animals were not reported.

3. **Methods:** A 0.5 mL volume of the undiluted test material was applied to an intact, previously clipped, 6-cm^2 application site on the dorsal trunk of each rabbit and covered with a 1 inch by 1 inch, 4-ply gauze patch held in place by 3-inch, semi-occlusive Micropore tape wrapped around the torso. The patch and any residual test material were removed after four hours of exposure. Application sites were scored for dermal irritation according to the Draize system at 30-60 minutes and 24, 48, and 72 hours and at 7, 10, and 14 days after patch removal. The study was conducted in stepwise fashion with testing of additional animals to follow, pending the absence of corrosive effects in an initial animal (#3501), which was treated at three application sites (for 3 minutes, 1 hour, or 4 hours).

II. RESULTS:

1. **Mortality:** There were no deaths or unscheduled humane sacrifices.

2. **Dermal responses:** The individual and mean erythema and edema scores are given in Table 1. At 30-60 minutes after patch removal, two animals had well-defined erythema (score=2), and one animal had very slight, barely perceptible erythmema (score=1), and two animals had very slight or slight edema (score=1-2). At 24 hours, all three animals had well-defined erythema with slight edema. At 48 and 72 hours, two animals had moderate to severe erythema (score=3), one animal had well-defined erythema, and all three animals had slight edema. Thereafter, the erythema and edema generally lessened in incidence and severity until all three animals were free of these findings at 14 days after patch removal. Additional observations included light brown areas on the dose sites of 1-2 animals between 24 hours and 10 days, desquamation at the dose sites of 2-3 animals between 7 and 14 days, and new skin at the dose sites of 1-2 animals between 10 and 14 days after patch removal.

[a] The humidity exceeded the stated upper limit for three days during the study

Irritation Scores:

TABLE 1. Summary of individual rabbit's dermal irritation scores with time (after patch removal)								
Animal No.	Sex	Hours				Days		
		0.5-1	24	48	72	7	10	14
3501[e]	F	1/0 [a]	2/2	3/2	3/2	2/1 [b]	1/0 [b]	0/0 [b]
3502	F	2/1	2/2 [c]	2/2 [c]	2/2 [c]	2/1 [b]	1/0 [b, d]	0/0 [d]
3503	F	2/2	2/2 [c]	3/2 [c]	3/2 [c]	3/2 [c]	1/0 [b, c]	0/0 [b, d]
Mean severity of Irritation		1.67/1.00	2.00/2.00	2.67/2.00	2.67/2.00	2.33/1.33	1.00/0.00	0.00/0.00
Total Primary Dermal Irritation (PDI) score (mean erythema + mean edema)		2.67	4.00	4.67	4.67	3.66	1.00	0.00
Primary Dermal Irriation Index (PDII) = PDI for 0.5-1 hr + 24 hr + 48 hr + 72 hr / 4							4.0	

Data taken from Table 2, p. 16, MRID 48275006.

[a] Erythema/Edema.

[b] Desquamation at the dose site.

[c] Light brown areas at the dose site.

[d] New skin at the dose site.

[e] Results for four hour exposure

DESCRIPTION OF RATING METHOD[a]

Evaluation of Skin Reactions: **Score**

Erythema and eschar formation:

No erythema 0
Very slight erythema (barely perceptible) 1
Well-defined erythema 2
Moderate to severe erythema 3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) 4

Edema Formation:

No edema 0
Very slight edema (barely perceptible) 1
Slight edema (edges of area well-defined by definite raising) 2
Moderate edema (raised approximately 1 mm) 3
Severe edema (raised by more than 1 mm extending beyond the area of exposure) 4

[a] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. There. 1944; 82:377-390.

III. DISCUSSION:

The primary dermal irritation index was 4.00, and 2/3 rabbits had moderate to severe erythema on the dose site at 72 hours. In agreement with the study author, the test material is a moderate irritant. The test material is placed in TOXICITY CATEGORY III. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

BM608
[TIMOREX GOLD - TEA TREE OIL]

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT [OCSPP 870.2400]
MRID 48275005

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Donna L. Fefee, AE
Date: JAN 04 2012

Secondary Reviewers:
Thomas C. Marshall, Ph.D., D.A.B.T.

Signature: Thomas C. Marshall, AE
Date: JAN 04 2012

Robert H. Ross, M.S., Program Manager

Signature: Robert H. Ross
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Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg
Date: JAN 04 2012

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DATA EVALUATION RECORD

BM608
[TIMOREX GOLD - TEA TREE OIL]

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT [OCSPP 870.2400]
MRID 48275005

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

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Donna L. Fefee, D.V.M.

Signature: _____
Date: _____

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Thomas C. Marshall, Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Program Manager

Signature: _____
Date: _____

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: _____
Date: _____

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DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales /s/ 1/24/13**
STUDY TYPE:	Acute Eye Irritation - Rabbits (OCSPP 870.2400)
MRID NO:	48275005
DP BARCODE NO:	396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	BM608 (Timorex Gold) (Batch # 6345)
PROJECT NO:	29831
SPONSOR:	Stockton Crop Protection AG, 112 Baarer Str. CH-6302, Zug, Switzerland
TESTING FACILITY:	Eurofins PSL, 2394 US Highway, 130 Suite E, Dayton, New Jersey 08810.
TITLE OF REPORT:	BM 608: Primary Eye Irritation Study in Rabbits
AUTHOR:	Jennifer Durando, B.S.
STUDY COMPLETED:	September 2, 2010
GOOD LABORATORY PRACTICE:	Study meets requirements of 40 CFR Part 160; specific information related to the characterization of the test material was the responsibility of the sponsor.
CONCLUSION:	Moderately irritating (per Kay and Calandra) in both rinsed and unrinsed eyes.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY II

I. STUDY DESIGN:

1. **Test Material:** BM 608; nominal composition: 23.14% Tea Tree Oil (a.i.) and 76.86% other ingredients; Batch No.: 6345; PSL Reference No.: 100426-8H; yellow-light brown liquid; pH: 9.15; soluble in water; expiration date: December 22, 2011; stored at room temperature and expected to be stable for the duration of testing.

2. **Test Animals:** Six young adult female New Zealand albino rabbits were received from Robinson Services, Inc. (Clemmons, N.C.), identified using stainless-steel ear tags (#3401-#3406), and acclimated for 13-20 days. Housing was individual in suspended stainless-steel cages with mesh floors. The animals were fed pelleted Purina Rabbit Chow #5326 and supplied with *ad libitum* filtered tap water. The environmental conditions of the animal room

were as follows: temperature, 19-22° C; relative humidity, 59-92%;[a] air changes, not reported; and photoperiod, 12 hour light/dark cycle. The exact ages and body weights of the animals were not reported.

3. **Methods:** Prior to initiation of the test, both eyes of each test animal were examined for pre-existing ocular irritation using a white light source and a fluorescein dye. Only healthy naïve animals without ocular irritation were used for the testing. A 0.1 mL volume of undiluted test material was instilled into the conjunctival sac of the anesthetized (2-3 drops of tetracaine hydrochloride ocular anesthetic) right eye of each animal, and the upper and lower lids were held shut for approximately one second. The left eye was anesthetized in like fashion and served as a control. Eyes were scored for ocular irritation according to the Draize method 1, 24, 48 and 72 hours and at 4, 7, and/or 10 and 14 days after test material instillation, and sodium fluorescein staining was done at 24 hours (and, if warranted, at subsequent intervals). The treated eyes of three animals were washed with approximately 30 mL of physiological saline approximately 30 seconds after instillation of the test material. The study was conducted in stepwise fashion with testing of four additional animals (two rinsed and two unrinsed), pending the absence of corrosive effects in two initial animals (one rinsed and one unrinsed).

II. RESULTS:

1. **Mortality:** All rabbits survived the study.

2. **Ocular Lesions:** Observations are summarized in Tables 1a (cornea), 1b (iris), and 1c (conjunctiva), and the Total Irritation scores and Average Primary Irritation scores are given in Table 3.

   All unrinsed treated eyes had corneal opacity (score=1), iritis (score=1), and conjunctival redness, chemosis, and discharge (scores=3, 2, and 3, respectively) at one hour post-instillation. The incidence and severity of irritation decreased with time. In the unrinsed eyes, the corneal opacity resolved by 4-14 days, and the iritis resolved by 72 hours to 7 days post-instillation. All unrinsed treated eyes were free of "positive" conjunctival findings by day 14. The maximum "Average Primary Irritation" score for the unrinsed eyes was 39.3 at 1 hour after test material instillation, and all eyes were clear (Total Irritation Score=0) within 14 days.

   All rinsed treated eyes had corneal opacity (score=1), iritis (score=1), and conjunctival redness, chemosis, and discharge (scores=2-3, 2, and 3, respectively) at one hour post-instillation. The incidence and severity of irritation decreased with time. In the rinsed eyes, the corneal opacity resolved by 24-48 hours, and the iritis resolved by 48-72 hours post-instillation. All rinsed treated eyes were free of "positive" conjunctival findings by 7 days. The maximum "Average Primary Irritation" score for the rinsed eyes was 29.7 at 1 hour after test material instillation, and all eyes were clear (Total Irritation Score=0) within 7 days.

[a] The humidity exceeded the upper limit for two days during the study and a dehumidifier was used during those two days (MRID 48275005, page 9 of 21)

TABLE 1. Individual Eye Scores w/ Time: Cornea (A=Density of Opacity, B=Area of Opacity)[a]																
Animal No.	1 hour		24 hours		48 hours		72 hours		4 Days		7 Days		10 Days		14 Days	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Unrinsed															
3401	1	4	1	4	1	3	1	1	0	4	0	4	nd	nd	nd	nd
3403	1	4	1	4	1	4	1	4	1	2	0	4	0	4	0	4
3404	1	3	1	3	1	3	1	3	1	1	1	1	1	1	0	4
	Rinsed															
3402	1	2	0	4	0	4	0	4	0	4	0	4	nd	nd	nd	nd
3405	1	2	1	1	0	4	0	4	0	4	0	4	0	4	0	4
3406	1	2	1	1	0	4	0	4	0	4	0	4	0	4	0	4

TABLE 1a. Individual Eye Scores w/ Time: Iris[a]								
Animal No.	1 hour	24 hours	48 hours	72 hours	4 Days	7 Days	10 Days	14 Days
	Unrinsed							
3401	1	1	1	0	0	0	nd	nd
3403	1	1	1	1	1	0	0	0
3404	1	1	1	1	1	0	0	0
	Rinsed							
3402	1	1	1	0	0	0	nd	nd
3405	1	1	0	0	0	0	0	0
3406	1	1	0	0	0	0	0	0

TABLE 1b. Individual Eye Scores w/Time: Conjunctiva[a]							
Time	Conjunctivae	Unrinsed			Rinsed		
		3401	3403	3404	3402	3405	3406
1 hour	Redness	3	3	3	2	2	3
	Chemosis	2	2	2	2	2	2
	Discharge	3	3	3	3	3	3
24 hours	Redness	3	3	3	2	2	2
	Chemosis	2	2	2	1	2	2
	Discharge	2	3	3	2	2	2
48 hours	Redness	2	2	2	1	1	1
	Chemosis	1	2	2	1	0	0
	Discharge	1	2	2	1	1	1
72 hours	Redness	1	2	2	0	1	0
	Chemosis	1	2	2	0	0	0

	Discharge	1	2	2	0	0	0
4 days	Redness	1	2	2	0	1	0
	Chemosis	1	2	2	0	0	0
	Discharge	0	1	2	0	0	0
7 days	Redness	0	0	0	0	0	0
	Chemosis	0	0	0	0	0	0
	Discharge	0	1	1	0	0	0
10 days	Redness	nd	0	0	nd	0	0
	Chemosis	nd	0	0	nd	0	0
	Discharge	nd	1	1	nd	0	0
14 days	Redness	nd	0	0	nd	0	0
	Chemosis	nd	0	0	nd	0	0
	Discharge	nd	0	0	nd	0	0

[a] Irritation score is based on Draize method; see scale for scoring ocular lesions below

Scale for Scoring Ocular Lesions[b]

Cornea

A. Opacity-degree of density (area most dense taken for reading)
- No opacity 0
- Scattered or diffuse area [of opacity], details of iris clearly visible 1*
- Easily discernible translucent areas, details of iris slightly obscured 2*
- Opalescent areas, no details of iris visible, size of pupil barely discernible 3*
- Opaque [cornea], iris invisible 4*

B. Area of cornea involved
- One quarter (or less), but not zero .....
- Greater than one quarter, but less than half 2
- Greater than half, but less than three quarters 3
- Greater than three quarters, up to whole area 4

Score = A x B x 5 Total Maximum Score = 80

Iris

A. Grades
- Normal 0
- Folds above normal, congestion, swelling, circumcorneal hyperemia or injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive) 1*
- No reaction to light, hemorrhage, gross destruction (any or all of these) 2*

Score = A x 5 Total Maximum Score = 10

Conjunctive

A. Redness: (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
- Vessels normal 0
- Vessels definitely injected above normal 1
- More diffuse, deeper crimson red, individual vessels not easily discernible 2*
- Diffuse beefy red 3*

B. Chemosis: lids and/or nictating membranes
- No swelling 0
- Any swelling above normal (includes nictitating membrane) 1
- Obvious swelling with partial eversion of lids 2*
- Swelling with lids about half-closed 3*
- Swelling with lids more than half closed 4*

C. Discharge
- No discharge 0
- Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
- Discharge with moistening of the lids and hairs just adjacent to lids 2
- Discharge with moistening of the lids and hairs, and considerable area around the eye 3

Score = (A + B + C) x 2 Total Maximum Score = 20

* Reaction indicates a positive effect.
[b] Draize, J.H., Woodward, G., and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 1944; 82: 377-390

TABLE 2. Summary of Total[a] and Primary Eye Irritation Scores with Time

Animal No.	1 hour	24 hours	48 hours	72 hours	4 Days	7 Days	10 Days	14 Days
	Unrinsed							
3401 (F)	41	39	28	13	4	0	0	0
3403 (F)	41	41	37	37	25	2	2	0
3404 (F)	36	36	32	32	22	7	7	0
Average scores[b]	39.3	38.7	32.3	27.3	17.0	3.0	4.5	0.0
	Rinsed							
3402 (F)	29	15	11	0	0	0	0	0
3405 (F)	29	22	4	2	2	0	0	0
3406 (F)	31	22	4	0	0	0	0	0
Average scores[b]	29.7	19.7	6.3	0.7	0.7	0.0	0.0	0.0

[a]Formula: Total Irritation Score = I + II + III, where,
I = Corneal Score = [Density (A) x Area (B)] x 5
II = Iris Score = Severity x 5
III = Conjunctival Score = [Erythema (A) + Chemosis (B) + Discharge (C)] x 2
[b]Average Primary Irritation = Sum of Total Irritation Scores ÷ 3

III. DISCUSSION:

The reviewer agrees with the study author that the test material was moderately irritating (per Kay and Calandra). Based on the persistence of corneal opacity through day 7 in one unrinsed eye, the test material is placed in TOXICITY CATEGORY II. The packet classification is **ACCEPTABLE.**

IV. DEFICIENCIES:

The relative humidity in the animal room was as high as 92% for two days of the study, and a portable dehumidifier was used to lower the humidity level. This did not result in any apparent adverse effects on the animals or the study results.

DATA EVALUATION RECORD

TEA TREE OIL
[TEA TREE OIL TECHNICAL]

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT (OPPTS 870.1300)
MRID 48598701

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer:
Thomas C Marshall, PhD, DABT

Signature: Thomas C. Marshall, AE
Date: JAN 0 4 2012

Secondary Reviewers:
Donna L. Fefee, D.V.M.

Signature: Donna L. Fefee, AE
Date: JAN 0 4 2012

Robert Ross, M.S., Program Manager

Signature: Robert N. Ross
Date: JAN 0 4 2012

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg
Date: JAN 0 4 2012

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This review may have been altered subsequent to the contractor's signatures above.

Summitec Corp. for the U.S. Environmental Protection Agency under Contract No.EP-W-11-014

THIS DATA EVALUATION RECORD HAS BEEN ANNOTATED WITH PEER REVIEW COMMENTS FROM MICROBIAL AND BIOCHEMICAL EVALUATION SECTION (MBES)/PMRA (MARCH 27, 2012)

DATA EVALUATION RECORD

TEA TREE OIL
[TEA TREE OIL TECHNICAL]

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT (OPPTS 870.1300)
MRID 48598701

Prepared for
Biopesticides and Pollution Prevention Division
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Primary Reviewer:
Thomas C Marshall, PhD, DABT — Signature: _____ Date: _____

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Donna L. Fefee, D.V.M. — Signature: _____ Date: _____

Robert Ross, M.S., Program Manager — Signature: _____ Date: _____

Quality Assurance:
Jennifer Goldberg, B.S. — Signature: _____ Date: _____

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DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales /s/ 1/24/13**
STUDY TYPE:	Acute Inhalation Toxicity - Rats (OPPTS 870.1300)
MRID NO:	48598701
DP BARCODE NO:	DP 396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	Tea Tree Oil Technical (EPA Reg. No. 86182-E, five a.i.s (α-terpinene, γ-terpinene, terpinen-4-ol, cineole, *p*-cymene) comprise ~71.3% of the product.
PROJECT NO:	G7151
SPONSOR:	Stockton Crop Protection Ag, Baarestrasse 112, 6302 Zug, Switzerland
TESTING FACILITY:	Advinus Therapeutics Ltd., Dept. Safety Assessment, Toxicology, POB 5813, Lot Nos. 21 & 22, Peenya II Phase, Bangalore 560 058, India
TITLE OF REPORT:	Tea Tree Oil Technical - Acute Inhalation Toxicity Study in Wistar Rats
AUTHOR:	S.B. Mohan Kumar
STUDY COMPLETED:	November 9, 2010
GOOD LABORATORY PRACTICE:	Not in compliance with 40 CFR Part ; study conducted "in accordance with" OECD C(97) 186/Final
CONCLUSION:	The inhalation LC_{50} for males, females combined was 3.64 mg/L.
CLASSIFICATION:	**ACCEPTABLE- Note 1/24/13: Deficiencies have been addressed in the June 29, 2013 submission from the applicant in MRID 48878201. The data are ACCEPTABLE; the test substance is classified into Toxicity Category IV.**

I. STUDY DESIGN:

1. **Test Material:** Tea Tree Oil (CAS No. 68647-73-4), Batch No. XK-23, five a.i.s comprise ~71.3% of the product (α-terpinene, 9.13%; γ-terpinene, 18.45%; terpinen-4-ol, 40.50%; cineole, 1.55%; *p*-cymene, 1.66%).[1] No description of the remainder was provided.

2. **Test Animals:** Four groups of five male and five female Wistar rats (HsdCpb: WU) were obtained from in-house, random bred stock at the testing facility. The rats were 11-12 weeks of age on the day of exposure (day 1), weighed 270-298 g (males) and 164-200 g (females). Prior to and after exposure, the rats were housed individually in polysulfone cages with sterilized paddy husk bedding. The animals were fed rat/mice pelleted feed (Provimi Animal Nutrition India). Purified well water was available *ad libitum*. The environmental conditions of the animal room were: temperature, 20-23 °C; relative humidity, 64-67%; photoperiod, 12 hour light/dark cycle; and air changes, 12-15 per hour.

3. **Methods:** The rats were acclimated for 5-8 days prior to exposure. A range-finding test was conducted on three male and three female rats to assist in dose selection for the main study. The animals were exposed to the concentrations shown in Table 1. The rats were exposed nose-only in an inhalation chamber for four hours (day 1). Design of the exposure apparatus did not allow for observation of the animals during exposure. The rats were examined upon removal from the chamber, and at least once daily thereafter for 14 days. They were weighed prior to test material exposure and on days 3, 8, and 15. All rats were sacrificed and necropsied on day 15.

[1] The certificate of analysis of the test item from the test facility indicates the α-Terpinene, cineole, γ-Terpinene, p-cymene, and Terpinen-4-ol concentrations to be 9.13%, 1.55%, 18.45%, 1.66%, and 40.50%, respectively. The corresponding concentrations from the certificate of analysis from the study sponsor are 9.45%, 2.67%, 21.04%, 2.35%, and 37.98%, respectively.

TABLE 1. Concentrations, exposure conditions, mortality/animals treated								
Nominal Conc. (mg/L)	Analyzed Conc. (mg/L)	Particle Size ± SD (µm)[a]	Particles ≤3.3 µm (%)	Temp (°C)	Humidity (%)	Mortality		
						Male	Female	Combined
Range-finding Study								
Not provided (50% w/v in DMSO)	4.54 ± 0.32	0.97 ± 0.61	Not provided	Not provided	Not provided	2/3	1/3	3/6
Main Study								
0 (DMSO only)	0	1.60±1.11	~90	23.4 - 23.8	65.05 – 66.14	0/5	0/5	0/10
1.23 – 1.32 (30% w/v in DMSO)	0.77±0.1	0.82±0.42	>99	22.9 - 23.3	65.45 – 65.98	0/5	1/5	1/10
3.50 – 3.75 (50% w/v in DMSO)	3.69± 0.41	0.84±0.57	~97	22.2 - 22.7	67.42 – 69.38	2/5	2/5	4/10
5.88 – 7.07 (70% w/v in DMSO)	5.06± 1.13	0.74±0.33	>99	21.8 - 22.5	67.23 – 69.38	3/5	4/5	7/10

[a] Measured by particle size analyzer (GALAI-CIS-50)
Data taken from pp. 22-28, 32-37, 40-47, and 68 of MRID 48598701.

Generation of the test atmosphere and description of the chamber: The liquid test material was diluted in dimethyl sulfoxide (DMSO; w/v ratios presented in Table 1) and the exposure atmospheres were generated using a glass atomizer (source not named; figure without dimensions provided). Filtered compressed air was supplied to the atomizer using 1.6 kg/cm^2 of pressure at a rate of 0.4 mL/min. Additional diluent air was supplied directly to the exposure chamber to maintain a continuous airflow of 20 L/min. The nose only exposure chamber was comprised of two stainless steel cylindrical chambers, the aerosol exposure chamber with 24 exposure ports arranged in 4 tiers and the aerosol exhaust chamber. The rats were housed in restraining polycarbonate tubes during exposure. Temperature and humidity where the animals were housed was controlled and monitored. Time to equilibrium was approximately 2-6 minutes.

Test atmosphere concentration: Aerosol samples for measuring exposure concentrations were drawn through a silica gel column (10 L/min for 10 min.) connected to an exposure port four times at hourly intervals during the 4-hour exposure period. The test item concentrations in the exposure chamber were determined by analyzing the amount collected on the silica gel using a "validated analytical method" (not described). The results for the main study presented in Table 1 above are summarized from Table 5 in the MRID. Results from validation studies were also provided. The analytical method measured α-terpinene, γ-terpinene, and terpinen-4-ol, which are referred to as three of the five active ingredients in

Tea Tree Oil Technical in the Certificate of Analysis (COA) provided by the Sponsor (Appendix 3, p.). The concentration of the test material in the exposure chamber was estimated by normalizing each of the three analytes to their respective concentration provided on the COA (Amendment No. 1). This seems reasonable, but no justification was provided to confirm that these measurements are appropriate representations of the test material. The five active ingredients comprise about 71.3% of the test material according to the COA and the three measured active ingredients comprise about 68.1% of the test material. The balance of the test material was not addressed.

Particle size determination: The aerosol particle size was analyzed three times during exposure (first, second, fourth hours) using a GALAI-CIS-50 particle size analyzer (Galai Pvt. Ltd., Israel). Aerosol samples were drawn from an exposure port and the analyzer provided data on mean particle size and standard deviation using laser technology and a time-of-transition algorithm. Results are in Table 1 above.

II. RESULTS:

1. **Mortality:** Mortality was 1/10, 4/10, and 7/10 in the groups of rats exposed to 0.77, 3.69, and 5.06 mg/L of the test material, respectively (Table 1). All rats survived the 14-day observation period in the vehicle control group. Deaths were observed on day 3 (1 female) at the low concentration, days 2 (1 female) and 3 (2 male, 1 female) at the mid concentration, and days 1 (1 male), 2 (1 male, 1 female), 3 (1 male, 2 female), and 4 (1 female) at the high concentration. The 4-hour acute inhalation lethal concentration (LC_{50}) value in rats (males and females combined) for Tea Tree Oil based on these results is 3.64 mg/L of air (calculated by Probit analysis).

2. **Clinical Observations:** No toxic signs were observed in the vehicle control group. Lethargy and nasal discharge were observed on day 1 in the low concentration group and one female had tremors. Lethargy and "dullness" persisted in some males and females in this group through day 3, after which no signs were observed for most rats, except for one male in which no signs were observed starting on day 4. In the mid concentration group, lethargy, nasal discharge, and tremors were observed most of the rats on day 1. Other observations on day 1 in some males and females were ataxia and urine soaked perineum, and one female demonstrated dyspnea. Lethargy and dullness were observed in both sexes on day 2 in this group and in one male on day 3. No signs were observed from day 4 onward. In the high concentration group, nasal discharge, tremors, ataxia, dyspnea, and slight salivation were common observations in both males and females on day 1 and one female had a perineum wet with urine. A high incidence of lethargy and dullness were observed in both sexes on day 2, and dyspnea was observed in several animals. Lethargy and dullness persisted in most males and females in this group through day 3. No adverse signs were observed from day 4 onward.

3. **Body Weight:** All surviving rats gained weight during the study. All rats that died actually lost weight when compared to their initial body weight.

4. **Gross Necropsy:** No gross abnormalities were noted at necropsy, except for one male rat with lung congestion in the high concentration group that died on the day of exposure.

III. DISCUSSION:

Mortality was 1/10, 4/10, and 7/10 in the rats exposed to 0.77, 3.69, and 5.06 mg/L of the test material, respectively. Lethargy, nasal discharge, tremors, and ataxia were common toxic signs attributed to the test material with tremors, dyspnea, and ataxia showing a dose-dependent incidence rate. All surviving rats gained weight during the study, while those that died actually lost weight. The study author calculated an inhalation LC_{50} for males and females combined of 3.64 mg/L. The packet classification is **NOT ACCEPTABLE (upgradeable)**, and for this reason the test material cannot presently be classified in an EPA Toxicity Category.

Note 1/24/13: Deficiencies have been addressed in the June 29, 2013 submission from the applicant in MRID 48878201. The data are ACCEPTABLE; the test substance is classified into Toxicity Category IV.

IV. DEFICIENCIES:

Justification is needed to confirm that measuring 3/5 of the active ingredients (68.1% of the product) is an appropriate representation of the test material concentration in the exposure chamber, especially since the balance of the product composition was not addressed. Two minor deficiencies are: volume of the exposure chamber is not provided, and identification of the equipment used to measure actual concentrations of the test material is not provided.

Note 1/24/13: Deficiencies have been addressed in the June 29, 2013 submission from the applicant in MRID 48878201. The data are ACCEPTABLE; the test substance is classified into Toxicity Category IV.

DATA EVALUATION RECORD

TEA TREE OIL
[TEA TREE OIL TECHNICAL]

STUDY TYPE: 90-DAY ORAL TOXICITY STUDY-RAT (870.3100; OECD 408)
MRID 48598702

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer:
Tom C. Marshall, PhD, DABT

Signature: Tom Marshall. AE
Date: JAN 0 4 2012

Secondary Reviewers:
Dennis M. Opresko, PhD

Signature:
Date: JAN 0 4 2012

Robert Ross, M.S., Program Manager

Signature:
Date: JAN 0 4 2012

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg
Date: JAN 0 4 2012

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Summitec Corp. for the U.S. Environmental Protection Agency under Contract No.EP-W-11-014

THIS DATA EVALUATION RECORD HAS BEEN ANNOTATED WITH PEER REVIEW COMMENTS FROM MICROBIAL AND BIOCHEMICAL EVALUATION SECTION (MBES)/PMRA (MARCH 27, 2012)

DATA EVALUATION RECORD

TEA TREE OIL
[TEA TREE OIL TECHNICAL]

STUDY TYPE: 90-DAY ORAL TOXICITY STUDY-RAT (870.3100; OECD 408)
MRID 48598702

Prepared for
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Robert Ross, M.S., Program Manager

Signature: _____
Date: _____

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: _____
Date: _____

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EPA SECONDARY REVIEWER: Angela L. Gonzales 1/24/13 /s/

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [gavage]-rat
OPPTS 870.3100 [§82-1a] (rodent)

PC CODE: Not available **DP BARCODE:** 396175

TEST MATERIAL (PURITY): Tea Tree Oil [testing laboratory assumed the test material was 100% pure; five a.i.s comprise 73.49% of the product in Batch No. XK-23 (α-terpinene, 9.45%; γ-terpinene, 21.04%; terpinen-4-ol, 37.98%; cineole, 2.67%; *p*-cymene, 2.35%). Percentages are assumed from a Certificate of Analysis that does not provide the units of measure. No description of the balance of the test material was provided.]

SYNONYMS: None

CITATION: Yogesha, B.N. (2011) Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats. Advinus Therapeutics Ltd., Dept. Safety Assessment, Toxicology, POB 5813, Lot Nos. 21 & 22, Peenya II Phase, Bangalore 560 058, India. Laboratory Study Number: G7153. June 6, 2011. MRID 48598702. Unpublished.

SPONSOR: Stockton Crop Protection Ag, Baarestrasse 112, 6302 Zug, Switzerland

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 48598702), Tea Tree Oil (assumed 100% pure based on analysis of five a.i.s; Batch No. XK-23) was administered to 10 Wistar rats/sex/dose by gavage at dosages of 0 (peanut oil), 30, 60 or 120 mg/kg/day. An additional 20 rats/sex/dose were included in the high dose group, and an additional 10 rats/sex/dose in the control group. Ten rats from the high dose group and 5 rats from the control group were sacrificed 14 days and 28 days after completion of the 90-day dosing period to determine the reversibility of any effects observed.

No mortality was observed in male rats, but two moribund females at the high dose were sacrificed during the treatment period (one on day 40, the other on day 41). Pale discoloration of the liver was observed in these two female rats, a finding that was microscopically associated with hepatocytic vacuolation. Salivation that persisted for about 30 minutes after dosing was observed at all dose levels with an incidence that increased in a dose-related manner. There was no compound-related effect on body weight/body weight gain. No toxicologically relevant hematological, clinical chemistry, or urinalysis findings were noted at any dose level. Some motor activity measurements (such as ambulatory counts) were statistically ($p \leq 0.05$) affected in both sexes of the high dose group, but the toxicological significance of these findings is questionable since there was no correlation with abnormal functional observations. Female rats showed statistically significant increases in liver to body weight ratios at the mid- and high dose levels (+11% and +9%, respectively), and a significant increase in ovary to body weight ratio in the 28-day high dose recovery group (+31%). No microscopic correlates were observed for these

organ weight findings in females. Flaccid testes, some with a small appearance, were observed in high-dose males (4/10 at the end of treatment; 2/10 after the 14-day recovery period; 8/10 after the 28-day recovery period). Absolute organ weight and organ-to-body-weight ratios were significantly reduced in the testes and epididymides of males in the high-dose 28-day recovery group. These findings were microscopically associated with degenerative changes in the seminiferous tubules (loss of germ cells, formation of multinucleated germ cells, presence of cell debris in tubular lumen, atrophic appearance, and sertoli cell vacuolation). The degenerative findings in the testes occurred at the same or higher rate of incidence with an overall greater degree of severity in the high dose animals in the 14-day and 28-day recovery groups. Sperm granulomas and cellular debris in the lumen (inflammation involving epididymal lumen and interstitium with the presence of large zones of spermatozoa surrounded by a zone of macrophages, giant cells and fibroblasts) were observed in the epididymides of high dose males at the end of the 90-day treatment period. The incidence of sperm granulomas peaked in the 14-day recovery group, but the incidence and severity of cellular debris in the epididymal lumen increased in the 14- and 28-day recovery groups. Minimal cell debris was observed in the lumen of the epididymides in 1/10 male rats dosed at 60 mg/kg/day. No treatment-related histological changes were observed in males dosed at 30 mg/kg/day. Statistically significant treatment-related effects on sperm motility, sperm morphology, and epididymal sperm counts were observed at the high dose level at the end of the treatment period and in the 14- and 28-day recovery groups. Sperm counts were significantly lower relative to controls in the mid-dose group, and there was a significant decrease in sperm motility and an insignificant increase in abnormal sperm at this dose level.

The lowest-observed-adverse-effect level (LOAEL) of Tea Tree Oil in rats was 60 mg/kg/day in males based on testicular toxicity, and 60 mg/kg/day for females based on increased liver to body weight ratios. The no-observed-adverse-effect level (NOAEL) is 30 mg/kg/day for males and 30 mg/kg/day for females.

This study in rats is Not Acceptable/Guideline, but upgradeable. It does not satisfy the guideline requirement for a 90-day study in rodents [OPPTS 870.3100); (OECD 408)], because homogeneity testing of the dosing preparations or a justification for the lack of such data was not provided.

UPDATE 1/24/13: Homogeneity data were submitted by the applicant in the June 29, 2013 submission in MRID 48878202. The study is ACCEPTABLE.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Tea Tree Oil

Description:	Colorless to pale yellow liquid
Lot/batch #:	XK-23
Purity:	Plant extract (100% assumed); five a.i.s comprise 73.49% of the product in Batch No. XK-23 (α-terpinene, 9.45%; γ-terpinene, 21.04%; terpinen-4-ol, 37.98%; cineole, 2.67%; *p*-cymene, 2.35%). Percentages are assumed from a Certificate of Analysis that does not provide the units of measure. No description of the balance of the test material was provided.
Compound stability:	Batch expiration date was January 7, 2011. Test material was stable for 24 hours at room temperature
CAS # of TGAI:	Not provided
Structure:	NA, plant extract

2. Vehicle: Peanut oil

3. Test animals:

Species:	Rat	
Strain:	Wistar HsdCpb: WU	
Age/weight at study initiation:	8 weeks/ Males: 223-263g; Females: 152-187g	
Source:	In-house random bred	
Housing:	2/sex/cage in suspended polysulfone cages with corn cob bedding	
Diet:	Ssniff rats/mice pellets Spezialdiatin GmbH (Germany), *ad libitum*	
Water:	Deep bore-well water treated with activated charcoal filter and UV rays in Aquaguard on-line water filter *ad libitum*	
Environmental conditions:	**Temperature:**	21° - 24° C
	Humidity:	65-68%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	12 Days	

B. STUDY DESIGN:

1. **In life dates:** Start: September 4, 2010; End: December 2 (males), December 3 (females), 2010; December 17 and December 31, 2010 for 14-day and 28-day "recovery" period animals.

2. **Animal assignment:** Animals were stratified by weight and randomly assigned to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Dose to animal (mg/kg/day)[1]	Dosage volume (ml/kg)	# Male	# Female
Control[2]	0	10	20[3]	20[3]
Low	30	10	10	10
Mid	60	10	10	10
High	120	10	30[4]	30[4]

[1] Individual dosages were prepared daily and adjusted based on the most recent body weights.
[2] Peanut oil was administered to controls.

[3] Includes 14- and 28-day recovery groups of five rats each.
[4] Includes 14- and 28-day recovery groups of ten rats each.

3. **Dose selection rationale:** Selection of dose levels was based on the results of a 28-day study.

4. **Preparation and analysis of test mixtures:** The vehicle used for dosing controls and preparation of test mixtures was peanut oil. The test material was weighed and mixed with the appropriate volume of vehicle to achieve target concentrations. Dose formulations were prepared daily (3, 6, and 12 mg/mL) and were analyzed for concentration and stability of the active ingredients (a.i.s) on day 1 and during months 2 and 3. The analytical method measured α-terpinene, γ-terpinene, and terpinen-4-ol, which are referred to as three of the five a.i.s in Tea Tree Oil in the Certificate of Analysis (COA) provided by the Sponsor (Appendix 13). The five active ingredients comprise about 73.5% of the test material according to the COA and the three measured active ingredients comprise about 68.5% of the test material. The approach seems reasonable, but no justification was provided to confirm that these measurements are appropriate representations of the test material.

Results:
Homogeneity analysis: The homogeneity and stability of the test material in the vehicle was carried out at 1.0 and 100 mg/mL in Advinus Study No. G7150. The test material was found to be stable for 24 hours in the vehicle at room temperature. No further details were provided.

Stability analysis: Analyses showed the test material was stable for 24 hours at room temperature.

Concentration analysis: The relative standard deviations of the measured a.i.s ranged from 1.55% to 6.88%, and the measurements were ≤16% of nominal in all analyzed preparations.

The reviewer considers the stability and concentration analyses acceptable, but either homogeneity data or a justification for the lack of such data needs to be provided.

Statistics: Body weights, net weight gains, food consumption, organ weights and their ratios were analyzed using predetermined statistical tests built into Provantis software (Version 8.0.1, Instem LSS, U.K.). Data collected and processed outside of the Provantis system were analyzed using an in-house developed and validated package in Excel or using SYSTAT (Version 12.0) and/or the inbuilt statistical analysis procedures in the Provantis™ software. Quantitative variables, such as haematology and clinical chemistry were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) within the group before performing a one-factor ANOVA modeling by treatment groups. When data were determined to be non-normal or heteroschedastic, ANOVA was conducted using suitable transformations. Comparison of means between treatment groups and the vehicle control group was done using Dunnet's 't' test following an 'F' test that was found to be significant. Recovery groups data were analyzed using Student's t-test. Comparison of means between the treatment recovery group and the control recovery group was performed. The minimum level of significance was $p \leq 0.05$.

C. METHODS:

1. **Observations:**

1a. **Cageside observations:** Animals were inspected twice daily for signs of toxicity and mortality.

1b. **Clinical examinations:** Detailed clinical examinations were performed on all animals prior to dosing and weekly during the study period.

1c. **Neurological evaluations:** The neurobehavioral status of each rat was evaluated on days 82 and 83 of the treatment period for the main groups and days 102 and 116 for the recovery groups. The motor activity of each rat was measured for 60 minutes (1 min. intervals in blocks of 10 min.) using an automated animal activity measuring system (Columbus Instruments, Ohio, USA) equipped with a computer analyzer. Stereotypic time (small movements), ambulatory time (large ambulatory movement), horizontal counts, and ambulatory counts were monitored. Functional observations were:

FUNCTIONAL OBSERVATIONS			
	Home cage prior to removal:		**Physiological:**
X	1) Vocalization	X	1) Body weight
X	2) Tremors	X	2) Body temperature
X	3) Convulsions	X	3) Appearance
		X	4) Skin/fur examination
		X	5) Respiration
	Autonomic function:		**Neuromuscular:**
			1) Time to first step
X	1) Lacrimation and salivation	X	2) Gait score
X	2) Eye examination/pupillary response	X	3) Mobility score
X	3) Palpebral closure	X	4) Landing hindlimb foot splay
X	4) Defecation	X	5) Forelimb grip strength
X	5) Urination	X	6) Hindlimb grip strength
X	6) Piloerection	X	7) Air righting
X	7) Perineum wetness		8) Rotorod
X	8) Tremors	X	9) Extensor thrust response
		X	10) Muscle tone
	Sensorimotor:		**CNS activity:**
		X	1) Posture
X	1) Tail pinch	X	2) Catalepsy
X	2) Startle response	X	3) Rearing
X	3) Touch response	X	4) Grooming
X	4) Approach response	X	5) Backing
	5) Olfactory response	X	6) Bizarre behaviour
	CNS excitability:		
X	1) Ease of removal		
X	2) Handling reactivity		
X	3) Clonic movements		
X	4) Tonic movements		
X	5) Arousal		
X	6) Vocalization		

2. **Body weight:** Animals were weighed weekly beginning day 1 prior to dosing. Mean body weight changes were calculated for each study week. A final (fasted) body weight was recorded for each animal on the day of sacrifice.

3. **Food consumption:** Food consumption was measured weekly during the study. Cagewise

mean food intake was calculated as g/animal/day.

4. **Ophthalmoscopic examination:** Eyes of all animals were examined prior to dosing and at the end of the treatment or recovery periods. Mydriasis was induced before examination using a solution of 1% Tropicamide.

5. **Hematology and clinical chemistry:** Blood was collected from all surviving animals at the end of the treatment or recovery periods for hematology and clinical chemistry; the animals were fasted overnight prior to each collection. Blood was collected by puncture of the retro-orbital sinus while the rats were under isoflurane anesthesia. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
X	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis*:** Each surviving rat was placed in a specially designed cage for urine collection at the end of the treatment or recovery periods. The following parameters were recorded:

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity / osmolality	X	Bilirubin
X	pH	X	Blood/blood cells
X	Sediment (microscopic)	X	Nitrite
X	Protein	X	Urobilinogen

7. **Sacrifice and pathology**: Each surviving rat and two that were sacrificed moribund were subjected to a gross pathology examination and the CHECKED (X) tissues were collected for histological examination. The collected tissues from all animals in the control and high dose groups were examined microscopically. Special emphasis was given to male and female reproductive organs (staging of spermatogenesis; detailed examination of interstitial cell structures in testes; subjective evaluation of follicles, corpora lutea, interstitial cell structures in ovaries and estrus cycle staging in uterus and vagina). Based on the microscopic findings observed in high dose males, kidneys, spleen, testes and epididymides were considered as target organs and were examined in males of the mid and low dose groups as well as the two recovery groups. All gross lesions from all rats were examined. The (XX) organs were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+	X	GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid (if present)*
X	Rectum*	X	Urinary bladder*	XX	Thyroid*
XX	Liver*+		Testis (right)*+	X	OTHER
		XX	Testes		
	Gall bladder (not rat)*		Epididymis (right)*+	X	Bone (sternum and/or femur)
		XX	Epididymides		
	Bile duct (rat)	XX	Prostate*	X	Skeletal muscle
X			Seminal vesicles*	X	
	Pancreas*	XX	Seminal vesicles with coagulating glands		Skin*
X	RESPIRATORY	XX	Ovaries *+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus (with cervix)*+		Coagulating gland
XX	Lung*	X	Mammary gland*		Vas deferens
	Nose*	X	Vagina		
	Pharynx*	X	Oviducts		
	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

treated females during the treatment period. Glucose was higher at the mid and high dose (+19 and +25%, respectively), as was sodium (+1%). Calcium (+11 to +17%) and chloride (+3 to +4%) were higher at all three doses. Elevated chloride (+2%) was observed in the 14- and 28-day female recovery groups, and decreased calcium (-4%) in the 14-female recovery group at the high dose. The slight nature of the increases and the lack of histological correlates imply that these differences are not treatment related.

F. URINALYSIS: No effects were observed in the urinalysis data.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** There were statistically significant compound-related effects in male rats on organ weights and organ weights relative to final body weight in the 28-day recovery group (Table 3). Microscopic findings correlated with the organ weight effects observed in the testes and epididymides, but no microscopic correlates were observed in the adrenal, thyroid, or parathyroid glands. Female rats showed statistically significant increases in liver to body weight ratios at the mid- and high dose levels (+11% and +9%, respectively), and a significant increase in ovary to body weight ratio in the 28-day high dose recovery group (+31%). No microscopic correlates were observed for these organ weight findings in females.

TABLE 3. Selected terminal organ weight data from male rats treated with Tea Tree Oil

	Mean organ weight (g)							
	0 mg/kg			30 mg/kg	60 mg/kg	120 mg/kg		
		Recovery					Recovery	
Organ	Main	14-Day	28-Day	Main	Main	Main	14-Day	28-Day
Testes	3.79	3.81	3.76	3.85	3.95	3.53	3.61	2.86* (-24%)
Epididymides	1.50	1.56	1.52	1.51	1.48	1.69	1.73	1.15* (-24%)
	Relative organ weight (g/100g body weight)							
		Recovery					Recovery	
	Main	14-Day	28-Day	Main	Main	Main	14-Day	28-Day
Testes	0.970	0.974	0.967	0.984	0.989	0.909	0.980	0.717* (-26%)
Epididymides	0.382	0.400	0.390	0.387	0.370	0.439	0.466	0.290* (-26%)
Adrenals	0.017	0.017	0.018	0.017	0.016	0.018	0.017	0.016* (-11%)
Thyroid + Parathyroids	0.007	0.008	0.008	0.007	0.008	0.008	0.009	0.010* (+25%)

Data taken from p. 292-320 of MRID 48598702.
* $p \leq 0.05$

2. **Gross pathology:** Flaccid testes, some with a small appearance, were observed in high-dose males (4/10 at the end of treatment; 2/10 after the 14-day recovery period; 8/10 after the 28-day recovery period). This finding was microscopically associated with degenerative changes in the seminiferous tubules. Abcesses were observed in the epididymides of all high dose group males (6/10 in main group, 4/10 in 14-day recovery group, and 1/10 in 28-day recovery group). Microscopically, the abscesses were sperm granulomas and the incidences in the recovery groups appear to indicate partial recovery. Pale discoloration of the liver was observed in one high dose recovery female rat sacrificed in moribund condition on day 40 of

treatment. This finding was microscopically associated with hepatocytic vacuolation. No abnormal findings were observed in the low- or mid-dose rats.

3. **Microscopic pathology:** Treatment-related microscopic lesions were primarily limited to the reproductive system of male rats (Table 4). No treatment-related histological changes were observed in males dosed at 30 mg/kg/day. Minimal cell debris was observed in the lumen of the epididymides in 1/10 male rats dosed at 60 mg/kg/day. At 120 mg/kg/day, the following microscopic changes were observed in the testes of male rats at varying degrees of severity: degenerative changes in the seminiferous tubules with loss of germ cells, formation of multinucleated germ cells, presence of cell debris in tubular lumen and atrophic appearance, and sertoli cell vacuolation characterized by the presence of discrete and basally located pleomorphic vacuoles. These findings were also observed in male rats at the same or higher rate of incidence and an overall greater degree of severity in the high dose animals in the 14-day and 28-day recovery groups. Sperm granulomas and cellular debris in the lumen (inflammation involving epididymal lumen and interstitium with the presence of large zones of spermatozoa surrounded by a zone of macrophages, giant cells and fibroblasts) were observed in the epididymides of high dose males at the end of the 90-day treatment period. The incidence of sperm granulomas peaked in the 14-day recovery group, but the incidence and severity of cellular debris in the epididymal lumen increased in the 14- and 28-day recovery groups. Aspermia was observed in one male at the end of the dosing period, while the incidence of oligospermia increased in the 14- and 28-day recovery groups.

   Other microscopic findings in high dose males at the end of the treatment period were a minimal degree tubular dilatation in the corticular area of kidney observed in 3/10 rats, and a minimal degree of vacuolation in the red pulp area of the spleen observed in 5/10 rats. No adverse findings in the kidney or spleen were observed in rats from the 14- and 28-day recovery groups.

   In females there were no treatment-related microscopic findings except in the two high dose recovery rats which were sacrificed in moribund condition on days 40 and 41 of treatment. Adverse findings for these animals were observed in spinal cord (degenerative), liver (hepatocyte vacuolation and necrosis), kidney (vacuolation of tubular epithelium), adrenal glands (hypertrophy of zona fasciculata), thymus and spleen (lymphoid depletion), sternum and femur (bone marrow hypocellularity), heart (inflammatory foci in myocardium), and stomach (mucosal necrosis). These changes could be related to test item administration.

8. **Spermatogenic analysis:** Sperm from the right vas deferens was collected for evaluation of sperm motility (Hamilton - Thorne Tox Ivos sperm analyzer) and sperm morphology from all surviving males in each experimental group under isoflurane anaesthesia. In addition, the right epididymides samples collected at necropsy were frozen and cauda epididymal sperm counts were conducted.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Salivation that persisted for about 30 minutes after dosing was observed at all dose levels with an incidence that increased in a dose-related manner. Two moribund females at the high dose and sacrificed on treatment days 40 and 41 had the following signs: pupil dilation, salivation, abdominal respiration, weakness, hypoactivity, loss or impairment of limb function, gait problems, lacrimation, and piloerection. All observations were considered treatment-related. No clinical signs were observed during the 14-day or 28-day recovery periods.

2. **Mortality:** Two moribund females at the high dose were sacrificed (one on day 40, the other on day 41).

3. **Neurological evaluations:** No abnormalities were observed during home cage observations, handling, open field observations, sensory reactivity, or landing footsplay. There was a marginal increase of about 9% in the hindlimb grip strength in main group males at all dose levels. This was considered incidental as there was no dose-response relationship in the mean values and no noticeable changes in muscle tone during the handling observations. Statistically significant decreases ($p \leq 0.05$) in stereotypic activity time was observed in mid- and high-dose females, while a significant increase in horizontal counts was observed in high-dose males. However, ambulatory time significantly decreased in both the 14-day and 28-day male recovery groups. The 14-day recovery group males also demonstrated significant decreases in stereotypic activity time, ambulatory counts, and horizontal counts. The 14-day recovery group females demonstrated significant decreases in ambulatory time, ambulatory counts, and horizontal counts. The toxicological significance of these motor activity findings, if any, is questionable since there is no correlation with abnormal functional observations.

B. BODY WEIGHT AND WEIGHT GAIN:

There was not a compound-related effect on body weight/body weight gain (Table 2). Statistically significant ($p \leq 0.05$) increased or decreased body weight gain were observed sporadically in treated males and females during the treatment and recovery periods, but these were not treatment related.

TABLE 2. Selected mean body weights and cumulative body weight gains during 90 days of treatment						
Dose rate [mg/kg/day]	Body weights (g ± SD)				Total weight gain	
	Day 1	Day 22	Day 50	Day 90	g	% difference from control[1]
Males						
0	240±12.1	313±18.3	372±25.2	408±28.6	+168	
30	241±12.8	310±17.1	364±18.2	408±20.7	+167	-0.6%
60	240±11.6	314±22.6	368±28.4	417±36.0	+177	+5.4%
120	238±12.1	302±13.2	359±16.3	403±21.0	+165	-1.8%
Females						
0	171±7.2	206±12.2	230±14.4	244±13.4	+73	
30	169±9.8	206±11.3	231±14.3	245±15.5	+76	+4.0%
60	169±10.4	201±14.7	225±15.3	239±18.0	+69	-5.5%
120	169±11.3	205±13.0	228±12.9	240±13.8	+72	-1.4%

Data obtained from pages 88-102 of MRID 48598702 .
[1]Calculated by reviewer.

C. FOOD CONSUMPTION:

1. **Food consumption:** Statistically significant decreases in the weekly interval food consumption were slight but consistent among males in the low and high dose group. This trend continued throughout the recovery period, although food consumption increased for both the control and treated groups. Statistically significant decreases in weekly food consumption were observed for females in the low and high dose groups and recovery group females for days 15 to 22, but recovery group females on days 29-36, 50-57, and 97-104 had statistically significantly increased food intakes. These findings are not considered toxicologically significant as body weights were not markedly affected.

2. **Compound consumption:** NA (not a feeding study).

3. **Food efficiency:** Not reported

D. OPHTHALMOSCOPIC EXAMINATION: No compound-related effects on the eyes were observed.

E. BLOOD ANALYSES:

1. **Hematology:** There was no compound-related effect on the hematology of treated rats. A few statistically significant ($p \leq 0.05$) increased or decreased parameters were observed sporadically in high-dose main study and recovery treated males and females during the treatment and recovery periods, but these were not treatment related.

2. **Clinical chemistry:** There were statistically significant ($p \leq 0.05$) increased or decreased parameters in treated males during the treatment and recovery period, but these were minimal, random changes that were considered to be incidental and not compound-related. Statistically significant ($p \leq 0.05$) increased or decreased parameters were also observed in

TABLE 4. Selected histopathology findings in male rats treated with Tea Tree Oil

	Incidence and Severity by Dose Level and Group							
	0 mg/kg			30 mg/kg	60 mg/kg	120 mg/kg		
		Recovery					Recovery	
Organ/Lesion	Main	14-Day	28-Day	Main	Main	Main	14-Day	28-Day
Testes [# rats]	[10]	[5]	[5]	[10]	[10]	[10]	[10]	[10]
Degenerative seminiferous tubules	0	0	0	0	0	8 (4@+; 2@2+; 1@3+; 1@4+)	9 (3@+; 4@2+; 2@3+)	8 (2@+; 4@2+; 2@3+)
Sertoli cell vacuolation	0	0	0	0	0	9 (7@+; 2@2+)	10 (6@+; 4@2+)	9 (5@+; 4@2+)
Sperm stasis	0	0	0	0	0	0	4 (4@+)	0
Epididymides [# rats]	[10]	[5]	[5]	[10]	[10]	[10]	[10]	[10]
Sperm granuloma	0	0	0	0	0	4	6	1
Lumen debris	0	0	0	0	1 (1@+)	7 (6@+; 1@2+)	9 (1@+; 6@2+; 1@3+; 1@4+)	9 (2@+; 5@2+; 2@3+)
Oligospermia	0	0	0	0	0	3	5	6
Aspermia	0	0	0	0	0	1	0	0
Kidneys [# rats]	[10]	[5]	[5]	[10]	]10]	[10]	[10]	[10]
Tubule dilation	0	0	0	0	0	3 (3@+)	0	0
Spleen [# rats]	[10]	[5]	[5]	[10]	[10]	[10]	[10]	[10]
Vacuolation	0	0	0	0	0	5 (5@+)	0	0

Data taken from p. 46-47, 330 of MRID 48598702.
+ = Minimal; 2+ = mild; 3+ = moderate; 4+ = marked

H. SPERMATOGENIC ANALYSIS: Statistically significant treatment-related effects on sperm motility, sperm morphology, and epididymal sperm counts were observed at the high dose level at the end of the treatment period and in the 14- and 28-day recovery groups (Table 5). Sperm counts were significantly lower relative to controls in the mid-dose group, and there was a significant decrease in sperm motility and an insignificant increase in abnormal sperm at this dose level. These findings are consistent with the histopathology data on testes and epididymides.

TABLE 5. Selected spermatogenic analysis data in rats treated with Tea Tree Oil								
	Observation by Dose Level and Group							
	0 mg/kg			30 mg/kg	60 mg/kg	120 mg/kg		
		Recovery					Recovery	
Organ/Lesion	Main	14-Day	28-Day	Main	Main	Main	14-Day	28-Day
Motility [# rats]	[10]	[5]	[5]	[10]	[10]	[10]	[10]	[10]
% Motile	84	83	83	85	65*	17*	31*	41*
Morphology [# rats]	[10]	[5]	[5]	[10]	[10]	[10]	[10]	[10]
% Abnormal	1.1	0.6	2.2	2.1	22	86*	84*	78*
Counts [# rats]	[9]	[5]	[5]	[10]	[10]	[10]	[10]	[10]
Number (10^6)/g cauda epididymis	771	804	728	687	605*	457*	354*	311*

Data taken from p. 44, 321-323 of MRID 48598702.
* $p \leq 0.05$

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS CONCLUSIONS:** The investigators stated that based on the results of this 90-day study (90 days for males and 91 for females) the No Observed Adverse Effect Level (NOAEL) of Tea Tree Oil in Wistar rats is 30 mg/kg/day for males and 60 mg/kg/day for females.

B. **REVIEWER COMMENTS:** The reviewer concurs with the investigators' conclusions. Marked testicular toxicity was observed in male rats administered 120 mg/kg/day for 90 days via gavage dosing, and this toxicity persisted or worsened in the 14- and 28-day recovery groups. Minimal cell debris was observed in the lumen of the epididymides in 1/10 male rats dosed at 60 mg/kg/day, and sperm counts were significantly lower at this dose level. Two female rats at the high dose level were sacrificed in moribund condition on days 40 and 41 of treatment. Adverse microscopic findings were observed in these two rats in spinal cord, liver, kidney, adrenal glands, thymus, spleen, bone marrow, and stomach. Increased liver to body weight ratios were observed in females at the 60 mg/kg/day dose level. No effect was observed in females at the 30 mg/kg/day dose level, and no effect was observed in males at 30 mg/kg/day.

The lowest-observed-adverse-effect level (LOAEL) of Tea Tree Oil in rats was 60 mg/kg/day in males based on testicular toxicity, and 60 mg/kg/day for females based on increased liver to body weight ratios. The no-observed-adverse-effect level (NOAEL) is 30 mg/kg/day for males and 30 mg/kg/day for females.

C. **STUDY/REPORT DEFICIENCIES:**

One major deficiency was that homogeneity testing of the dosing preparations or a justification for the lack of such data was not provided. A deficiency of less concern is that the chemical

analytical method measured three of the five a.i.s in Tea Tree Oil, but no justification was provided to confirm that these measurements are appropriate representations of the test material. The report would be strengthened by this information. A possible typographical error is the reference to one of the active ingredients as "cincole", when the appropriate name may be "cineole".

UPDATE 1/24/13: Homogeneity data were submitted by the applicant in the June 29, 2013 submission in MRID 48878202. The study is ACCEPTABLE.

DATA EVALUATION RECORD

Tea Tree Oil Technical

STUDY TYPE: Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OCSPP 850.1010)

MRID 48598703

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee
Task #4, Work Order No. 11-024

Primary Reviewer:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong, ME
Date: JAN 0 4 2012

Secondary Reviewers:
Dennis M. Opresko, Ph.D.

Signature:
Date: JAN 0 4 2012

Robert H. Ross, M.S., Program Manager

Signature:
Date: JAN 0 4 2012

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg
Date: JAN 0 4 2012

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

DATA EVALUATION RECORD

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Robert H. Ross, M.S., Program Manager

Signature: _____
Date: _____

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: _____
Date: _____

Disclaimer

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DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales /s/ 1/24/13**
STUDY TYPE:	Freshwater Aquatic Invertebrate Testing, Tier I (OPPTS 885.4240)
MRID NO:	48598703
DP BARCODE:	396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	Tea Tree Oil Technical
STUDY NO:	DAI13977
SPONSOR:	Biomor Israel Ltd.
TESTING FACILITY:	Dr.U.Noack-Laboratorien, Kathe-Paulus-Str.1, 31157 Sarstedt, Germany
TITLE OF REPORT:	Acute Immobilization Test to *Daphnia magna*, Semi-static, 48 h
AUTHOR:	Martina Noack
STUDY COMPLETED:	April 4, 2011
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The study was not conducted in compliance with 40 CFR Part 160 but was conducted in accordance with OECD Good Laboratory Regulations (OECD C(97) 186/Final).
STUDY SUMMARY:	A laboratory study was conducted to assess the acute toxicity of Tea Tree Oil (Batch XK-19; active ingredients identified were: 9.95% α-Terpinen; 22.81% γ-Terpinen; 4.62% Cineole; 1.89% p-Cymene; 40.7% Terpinen-4-ol; totaling 79.97% (no other ingredients identified) to the aquatic invertebrate, *Daphnia magna*. In a 48-hour semi-static water renewal study, daphnids were exposed to Tea Tree Oil mean measured concentrations of 0.106, 0.169, 0.374, 0.750, 1.60 and 2.65 mg/L. Based on animal immobilization, the Tea Tree Oil 48 h EC_{50} for *Daphnia magna* was estimated to be 0.591 mg/L (95% confidence limits : 0.499-0.700 mg/L). The NOEC after 48 h was 0.106 mg/L the highest test concentration with no biologically significant effect. The LOEC after 48 h was 0.169 mg/L the lowest test concentration with a biologically

significant effect. Validity criteria for this daphnid immobilization study were fulfilled according to OECD Guideline 202; there was less than 10% immobilization of animals in the control group and the dissolved O_2 concentration was ≥3 mg/L after 48 h.

CLASSIFICATION: **Acceptable.**

Test Material

The test material is Tea Tree Oil, Batch XK-19 (9.95% α-terpinen; 22.81% γ-terpinen; 4.62% cineole; 1.89% p-cymene; 40.7% terpinen-4-ol (no other ingredients listed). A certificate of analysis is provided on p. 39 of MRID 48598703. The expiration date of the test material was 11.19.2011. After receipt at the test facility, the test material was maintained at room temperature and protected from light.

Test Methods

A laboratory study was conducted to assess the acute toxicity of Tea Tree Oil Technical to the aquatic invertebrate, *Daphnia magna* STRAUS (Clone 5). The source of the daphnids was in-house cultures maintained at the test facility. Daphnids were grown in glass vessels in Elendt M4 media with a hardness of 160 to 180 mg $CaCO_3$/L. Daphnids were fed at least 5 times per week a mixture of unicellular green algae.

The test material, Tea Tree Oil (Batch XK-19), was freshly prepared with dilution water at the beginning of the test (0h) and at the water renewal (24h). The test concentrations were 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg/L corresponding to the geometric mean measured concentrations of 0.106, 0.169, 0.374, 0.750, 1.60 and 2.65 mg/L. The negative control was dilution water only and the positive control was potassium dichromate prepared at concentrations of 0.625, 1.00, 1.60 and 2.56 mg/L.

Dilution water was ISO test water prepared according to OECD 202 Annex 3 which consisted of;

KCl	5.76 mg/L
$NaHCO_3$	64.8 mg/L
$CaCl_2$	294 mg/L
$MgSO_4$	123 mg/L
pH	7.8±0.2

The protocol for daphnid immobilization testing was semi-static with water renewal after 24 hours and the study duration was 48 hours. The test vessels were 60 mL glass flasks which were filled with the test material, negative control, and positive control solutions and having nearly no headspace to reduce loss of test solutions. Twenty daphnids, divided into 4 replicates, each with 5 individuals per concentration level and test group were acclimated for 2 h before introduction into the test solutions.
Test vessels were maintained at 18-22°C in diffuse light with a 16/8 hour light/dark cycle. The daphnids were not fed during the study. Concentrations of the test material and positive control were analytically verified by GC-MS in samples collected from the fresh and old media. Water

quality parameters were measured at 0 h and at water renewal (24 h). Daphnid immobilization was monitored throughout the 48 hour test period and animals were considered immobile if unable to swim within 15 seconds of gentle agitation of the test vessel.

Statistical analyses were performed to determine EC_{t0} and EC_{50} values by sigmoidal dose-response regression. Confidence intervals for EC_{50} were calculated from best fit values and standard error and t-distribution calculated.

The validity criteria for an acceptable daphnid immobilization study requires that less than 10% of the control organisms be immobile in the 48 h test period and the dissolved O_2 concentration at the end of the test should be ≥3 mg/L.

Summary of Results

During the test, the room temperature ranged from 19 - 20°C and measured water quality parameters (pH, oxygen concentration, temperature, conductivity and hardness) are presented in Tables 1 -3. There were no abnormalities observed in the water quality parameters in the fresh and old test media.

Table 1. Water quality parameters at 0 and 24 h (Fresh Media)

Nominal Test Item Concentration [mg/L]	0 h		24 h	
	pH-Value	Dissolved O_2-Concentration [mg/L]	pH-Value	Dissolved O_2-Concentration [mg/L]
8.00	7.67	8.65	7.78	8.63
4.00	7.69	8.66	7.79	8.85
2.00	7.67	8.37	7.79	8.66
1.00	7.69	8.38	7.81	8.68
0.500	7.70	8.30	7.88	8.71
0.250	7.70	8.33	7.85	8.71
Control	7.99	8.97	7.97	8.85

Table 2. Water quality parameters after 24 h (Old Media)

Nominal Test Item Concentration [µg/L]	pH-Values Replicates				Dissolved O_2 -Concentration [mg/L] Replicates			
	1	2	3	4	1	2	3	4
8.00	7.67	7.59	7.61	7.62	8.22	8.15	8.14	8.04
4.00	7.61	7.61	7.57	7.59	8.14	8.15	8.12	8.10
2.00	7.62	7.62	7.63	7.60	8.24	8.27	8.26	8.08
1.00	7.60	7.58	7.61	7.61	8.20	8.17	8.32	8.08
0.500	7.59	7.58	7.60	7.60	8.20	8.31	8.26	8.20
0.250	7.64	7.62	7.61	7.60	8.39	8.33	8.37	8.27
Control	7.56	7.67	7.68	7.68	8.54	8.50	8.53	8.55

Table 3. Water quality parameters after 48 h (Old Media)

Nominal Test Item Concentration [µg/L]	pH-Values Replicates				Dissolved O_2-Concentration [mg/L] Replicates			
	1	2	3	4	1	2	3	4
8.00	7.59	7.59	7.58	7.61	7.91	8.03	8.06	8.07
4.00	7.57	7.57	7.54	7.60	7.97	7.93	7.79	7.89
2.00	7.54	7.55	7.58	7.50	7.97	7.99	8.15	7.98
1.00	7.56	7.56	7.55	7.55	8.05	8.03	8.02	7.93
0.500	7.59	7.58	7.57	7.57	8.18	8.04	8.03	8.06
0.250	7.61	7.58	7.54	7.59	8.06	8.01	8.05	7.89
Control	7.92	7.89	7.66	7.69	8.36	8.35	8.35	8.17

The concentrations of the test item Tea Tree Oil were analytically verified by GC-MS in the fresh media (0 and 24 h) and old media (24 and 48 h). Recovery rates of the test item Tea Tree Oil in the old media (24 and 48 h) of all concentration levels were in the range of 76 to 157 % of the initially measured values in the fresh media at 0 and 24 h (Table 4). Therefore, it can be concluded that the chosen method for preparation of the test solutions leads to stable aqueous emulsions of the test item according to the OECD 202 guidance.

All effect values given are based on the geometric mean measured concentrations of Tea Tree Oil in the test solutions. The overall geometric mean measured concentrations of Tea Tree Oil were calculated to be: 0.106, 0.169, 0.374, 0.750, 1.60 and 2.65 mg/L, corresponding to overall recoveries in the range of 92 to 136%.

Table 4. Measured exposure concentrations of Tea Tree Oil in fresh and old test solutions.

Sampling date	2010-10-27 0 h (Fresh medium)	2010-10-28 24 h (Old medium)		2010-10-28 24 h (Fresh medium)	2010-10-29 48 h (Old medium)			
Start of analysis	2010-10-27	2010-10-28		2010-10-28	2010-10-29			
Nominal concentration of the test item [mg/L]	Tea Tree Oil						Overall geometric mean	
	Meas. conc. [mg/L]	Meas. conc. [mg/L]	RR [%]	Meas. conc. [mg/L]	Meas. conc. [mg/L]	RR [%]	Meas. conc. [mg/L]	RR [%]
8.00	2.76	2.92	106	2.13	2.89	136	2.65	120
4.00	1.58	1.50	95	1.48	1.87	126	1.60	110
2.00	0.777	0.864	111	0.644	0.732	114	0.750	112
1.00	0.315	0.377	120	0.328	0.504	154	0.374	136
0.500	0.174	0.133*	76	0.178	0.196	110	0.169	92
0.250	0.116	0.0932*	80	0.0858	0.135	157	0.106	112
Control	< LOQ	< LOQ		< LOQ	< LOQ			

Meas. conc = Measured concentration of test item, mean value of 2 injections
RR = Recovery rate related to the initially measured concentration in the fresh media (0 and 24 h)
LOQ = Limit of quantification of the *analytical method* (5 µg/L)
* = Reanalysis on 2010-10-29

Daphnid immobilization results for the Tea Tree Oil treatments are provided in Table 5. Based on the geometric mean measured concentrations of the test material, Tea Tree Oil, the 48 h EC_{50} was estimated to be 0.591 mg/L (95% confidence limits : 0.499-0.700 mg/L). The NOEC after 48 h was 0.106 mg/L the highest test concentration with no biologically significant effect. The LOEC after 48h was 0.169 mg/L the lowest test concentration with no biologically significant effect.

Table 5. Immobilization of *Daphnia magna* exposed to Tea Tree Oil after 24 h and 48 h exposure.

Tea Tree Oil Geometric Mean Measured Concentration of the Test Item [mg/L]	IMMOBILIZATION [%]									
	24 h					48 h				
	Replicates					Replicates				
	1.	2.	3.	4.	MV	1.	2.	3.	4.	MV
2.65	80	60	80	80	75	100	100	100	100	100
1.60	40	60	60	20	45	100	100	100	80	95
0.750	20	40	60	0	30	60	80	60	40	60
0.374	0	0	0	0	0	0	40	40	20	25
0.169	0	20	0	0	5	0	20	20	20	15
0.106	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

MV = Mean value

Validity criteria for this daphnid immobilization study were fulfilled according to OECD Guideline 202; there was less than 10% immobilization of animals in the control group and dissolved O_2 was ≥3 mg/L after 48 h. In addition, the EC_{50} for the daphnids in the reference control was 1.68 mg/L which is within the recommended range of 0.6-2.1 mg/L according to OECD Guideline 202.

Study Author's Conclusion

The study author concluded that the Tea Tree Oil 48 h EC_{50} for *Daphnia magna* was estimated to be 0.591 mg/L (95% confidence limits: 0.499-0.700 mg/L). The NOEC after 48 h was 0.106 mg/L the highest test concentration with no biologically significant effect. The LOEC after 48 h was 0.169 mg/L the lowest test concentration with a biologically significant effect.

Reviewer's Conclusion

The reviewer agrees with the study author's conclusion.

Note: The test material in this daphnid immobilization study was Tea Tree Oil (Batch XK-19); the percent composition of five of the active ingredients, totaling 79.97%, was reported). The submitted proposed label and CSF (dated 10/6/2010) is for Timorex Gold containing 23.80% Tea Tree Oil Technical. The test results support the data requirements for Tea Tree Oil, but may not be totally supportive of the registration of Timorex Gold, since 72.2% of the end use product consists of other ingredients whose effects on daphnids alone or in combination in the Timorex Gold formulation were not addressed.

DATA EVALUATION RECORD

TEA TREE OIL
(Tea Tree Oil Technical)

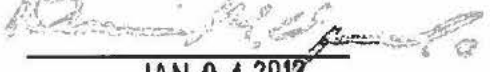
STUDY TYPE: Waiver Request
90-Day Inhalation Toxicity (OCSPP 870.3465)

MRID 48598704

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer: Thomas C. Marshall, Ph.D.	Signature: Date:	Thomas C. Marshall, AE JAN 04 2012
Secondary Reviewers: Dennis M. Opresko, Ph.D.	Signature: Date:	 JAN 04 2012
Robert Ross, M.S., Program Manager	Signature: Date:	 JAN 04 2012
Quality Assurance: Jennifer Goldberg, B.S.	Signature: Date:	Jennifer Goldberg JAN 04 2012

THIS DATA EVALUATION RECORD HAS BEEN ANNOTATED WITH PEER REVIEW COMMENTS FROM MICROBIAL AND BIOCHEMICAL EVALUATION SECTION (MBES)/PMRA (MARCH 27, 2012)

DATA EVALUATION RECORD

TEA TREE OIL
(Tea Tree Oil Technical)

STUDY TYPE: Waiver Request
90-Day Inhalation Toxicity (OCSPP 870.3465)

MRID 48598704

Prepared for
Biopesticides and Pollution Prevention Division
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One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer:
Thomas C. Marshall, Ph.D.

Signature: _____
Date: _____

Secondary Reviewers:
Dennis M. Opresko, Ph.D.

Signature: _____
Date: _____

Robert Ross, M.S., Program Manager

Signature: _____
Date: _____

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: _____
Date: _____

DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales /s/ 1/24/13**
STUDY TYPE:	Waiver Request 90-Day Inhalation Toxicity (OCSPP 870.3465)
MRID NO:	48598704
DP BARCODE:	DP396175
TEST MATERIAL:	Tea Tree Oil Technical (a.i., 100%; CAS No. not available)
STUDY NO:	None
SPONSOR:	Biomor Israel, Ltd. (EPA No. 86182)
TESTING FACILITY:	Not applicable
TITLE OF REPORT:	Supplemental Response to Tier 1 Biochemical Pesticide Data Requirements for Tea Tree Oil Technical
AUTHOR:	B.E. Mileson, PhD, DABT
STUDY COMPLETED:	August 5, 2011
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was provided. The document does not contain any data, and is therefore not subject to the requirements of 40 CFR Part 160.
CONCLUSION:	Sufficient information was provided to justify a waiver for 90-day inhalation toxicity testing of Tea Tree Oil Technical on the condition that the registrant provide verification that its manufacture and handling is conducted in a manner that substantially limits or eliminates inhalation exposure.

Product Description

Tea Tree Oil Technical was referred to as 100% active ingredient (a.i.) in the MRID. Two MRIDs (48598701 and 48598702) that accompanied this submission describe this plant-derived product as having five a.i.s that comprise 73.49% of the product in Batch No. XK-23 (α-terpinene, 9.45%; γ-terpinene, 21.04%; terpinen-4-ol, 37.98%; cineole, 2.67%; p-cymene, 2.35%). Percentages are

assumed from a Certificate of Analysis that does not provide the units of measure. No description of the balance of the test material was provided.

Waiver Request

The registrant is requesting a waiver from the requirement of conducting a 90-day inhalation toxicity study.

Registrant's Justification

Per 40 CFR Part 158.2050(e)(8), 90-Day Inhalation Toxicity data are required if there is a likelihood of significant levels of repeated inhalation exposure to the pesticide as a gas, vapor, or aerosol. Tea Tree Oil Technical is intended for further formulation into end-use products (Timorex Gold; 23.80% ai) that are diluted with water and applied as a foliar spray fungicide to growing crops. The proposed use pattern for Tea Tree Oil does not involve handling or application as a gas, vapor, or aerosol; consequently, no significant inhalation exposure is anticipated.

Tea Tree Oil Technical was low in acute toxicity to rats following a 4 hour acute inhalation exposure. An acute inhalation toxicity study with Tea Tree Oil Technical was conducted by nose-only exposure of male and female rats to aerosolized Tea Tree Oil Technical dissolved in dimethyl sulfoxide (MRID 48598701; review pending). Tea Tree Oil Technical concentrations determined analytically were: 0, 0.77, 3.69 and 5.06 mg/L of chamber air. Mean aerosol particle sizes were: 1.6, 0.82, 0.84, and 0.74 μm for each group, respectively. Nasal discharge, lethargy, dullness and tremors in some rats were among the signs of toxicity reported. The Tea Tree Oil Technical LC_{50} was 3.64 mg/L of air for both male and female rats. This indicates that Tea Tree Oil Technical is considered to be of low acute inhalation toxicity and classified as Toxicity Category IV for the inhalation route.

A guideline 90-Day Oral Toxicity study with Tea Tree Oil Technical was conducted that provided a no-observed-adverse-effect-level (NOAEL) of 30 mg/kg/day (MRID 48598702; review pending). Tea Tree Oil Technical was administered to rats by gavage at doses of 0, 30, 60 and 120 mg/kg/day for 90 days. Two satellite groups of rats were given the high dose or control treatment and kept after the main study to evaluate the potential recovery at 14 and 28 days post dosing. A Functional Observational Battery of tests indicated no neurobehavioral effects on open field behavior, handling behavior, or functional tests, only a possible increase in motor activity in high dose males and females compared to controls. Hematology, blood chemistry, and urine parameters were unchanged in treated rats compared to controls. At the end of the main study the only alteration in organ weights was an increase in the liver to body weight ratio in the females of the 60 mg/kg group and the 120 mg/kg group. At the end of the recovery phase, there was a significant decrease in the absolute and relative weights of the testes and epididymides of male rats sacrificed on day 29 post-dosing. There were statistically significant effects on sperm parameters in rats on the main study in the high dose group and in the 60 mg/kg/day group, but not in the 30 mg/kg/day group. The effects included a decrease in sperm motility, an increase in abnormal sperm, and a decreased sperm count. These effects corresponded to histopathologic changes in the testes and epididymides in the 120 mg/kg group, but not in the 60 mg/kg group. In the epididymides of the 120 mg/kg group males,

multiple abscesses were identified. There were no histopathologic effects in the pituitary, which suggests the effect on the male reproductive system is a direct effect rather than an effect on the hypothalamic-pituitary-gonadal axis. Effects on sperm parameters similar to those reported in the high dose main test group were observed in Tea Tree Oil Technical-treated rats of the recovery groups sacrificed 15 and 29 days after the dosing period ended, indicating that the rats had not fully recovered after the treatment. Indications of recovery were a time-dependent decrease in abnormal sperm (not evaluated for significance of trend) and a decrease in the incidence of epididymis abscesses. There was a clear NOAEL in this study of 30 mg/kg/day. Effects following repeated dose inhalation exposure are not expected to be different from those identified in the 90-day oral exposure.

Reviewer's Comments

Sufficient information was provided to justify a waiver for 90-day inhalation toxicity testing of Tea Tree Oil Technical on the condition that the registrant provide verification that its manufacture and handling is conducted in a manner that substantially limits or eliminates inhalation exposure. It should be noted that Tea Tree Oil Technical is intended to be diluted with water in end products and applied as a foliar spray fungicide to growing crops. Therefore, the statement that "use of the product does not involve handling as an aerosol" applies only to the technical grade product. Inhalation exposure will be an issue for end products. Agency guidance on waiver criteria for multiple exposure inhalation studies states that an applied aerosol product may be considered non-inhalable only if ≥99% of the particles exceed a particle size of 100 μm in diameter, otherwise properly conducted margin-of-exposure calculations need to be conducted (USEPA, 2002).

References

USEPA, 2002. SOP 2002.01 – HED Standard Operating Procedure: Guidance: Waiver criteria for multiple exposure toxicity studies. Attached to memo from M.J. Stasikowski dated August 15, 2002 to Health Effects Division staff.

DATA EVALUATION RECORD

TEA TREE OIL TECHNICAL

STUDY TYPE: WAIVER REQUEST
PRENATAL DEVELOPMENTAL TOXICITY - RAT [OCSPP 870.3700]

MRID 48598704

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task Order No. 4-11-024

Primary Reviewer:
Donna L. Fefee, D.V.M.

Signature: Donna L. Fefee, AE
Date: JAN 04 2012

Secondary Reviewers:
Thomas C. Marshall, Ph.D., D.A.B.T.

Signature: Thomas C. Marshall, AE
Date: JAN 04 2012

Robert H. Ross, M.S., Program Manager

Signature: Robert H. Ross
Date: JAN 04 2012

Quality Assurance:
Angela M. Edmonds, B.S.

Signature: Angela M. Edmonds
Date: JAN 04 2012

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

THIS DATA EVALUATION RECORD HAS BEEN ANNOTATED WITH PEER REVIEW COMMENTS FROM MICROBIAL AND BIOCHEMICAL EVALUATION SECTION (MBES)/PMRA (MARCH 27, 2012)

DATA EVALUATION RECORD

TEA TREE OIL TECHNICAL

STUDY TYPE: WAIVER REQUEST
PRENATAL DEVELOPMENTAL TOXICITY - RAT [OCSPP 870.3700]

MRID 48598704

Prepared for
Biopesticides and Pollution Prevention Division
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One Potomac Yard
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Robert H. Ross, M.S., Program Manager

Signature: _____
Date: _____

Quality Assurance:
Angela M. Edmonds, B.S.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales 1/24/13 /s/**
STUDY TYPE:	Waiver Request Prenatal Developmental Toxicity (OPPTS 870.3700)
MRID NO:	48598704
DECISION NO:	408891
DP BARCODE:	396175
TEST MATERIAL:	Tea Tree Oil Technical (a.i., 100.00% w/w tea tree oil)
PROJECT STUDY NO:	Not provided
SPONSOR:	Biomor Israel Ltd., PO Box 81, Qatzrin, 12900 Israel
TESTING FACILITY:	Technology Sciences Group, 1150 18th St. NW, Suite 1000, Washington, DC 20036
TITLE OF REPORT:	Supplemental Response to Tier I Biochemical Pesticide Data Requirements for Tea Tree Oil Technical
AUTHOR:	Beth E. Mileson, Ph.D., D.A.B.T.
STUDY COMPLETED:	August 5, 2011
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study does not meet the requirements of 40 CFP Part 160. There was no study director, and a quality assurance unit was not in place.
CONCLUSION:	Insufficient information was provided to support a waiver for a Prenatal Developmental Toxicity Study (OCSPP 870.3700). The provided study is **Non-guideline,** and it would not satisfy the *intent* of the guideline requirement for a developmental toxicity study with α-terpinene in the rat. However, because developmental toxicity was evident, this study could serve as a screening study and may provide useful supplemental information about the developmental toxicity of α-terpinene in rats. Insufficient information was provided to support the claim that α-terpinene is a reasonable surrogate for Tea Tree Oil Technical, especially since α-terpinene only represents approximately 10% of Tea Tree Oil.

Product Description

Tea Tree Oil Technical is a technical grade active ingredient (TGAI) intended for use in an end use product (Timorex Gold, 23.8% w/w tea tree oil) that will be diluted with water and applied to growing crops as a foliar spray fungicide. Tea tree oil is a natural product consisting primarily of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. The waiver request identifies the main components of tea tree oil as follows: terpinen-4-ol; γ-terpinene;

α-terpinene; 1,8-cineole; and p-cymene. All of these are cleared by the FDA for use as direct food additives (21 CFR 172.515, as cited in MRID 48598704).

Waiver Request

The registrant is requesting a waiver from the requirement to conduct a Prenatal Developmental Toxicity Study (OCSPP 870.3700).

Registrant's Justification

The data requirement can be met using existing data.

A prenatal developmental toxicity study was conducted using technical α-terpinene and is available in the published literature (Araujo; 1996). According to the registrant, α-terpinene is a reasonable surrogate for Tea Tree Oil Technical because it is one of the primary components of the TGAI and because it is structurally similar to four other primary components of the TGAI (specifically terpinen-4-ol, γ-terpinene, 1,8-cineole, and p-cymene) in that all five of these primary components are monocyclic monoterpines. The registrant also indicated that α-terpinene is a good surrogate because its chemical structure suggests that its metabolism and removal from the body will be representative of Tea tree oil technical, if not more problematic because it requires phase I metabolism first before being conjugated and excreted in the urine.

Copies of references were provided.

Reviewer's Comments

A full evaluation of the developmental toxicity study with α-terpinene in rats is provided in the Appendix. In this study, technical α-terpinene (89% a.i.) was administered to groups of 15-28 mated female Wistar rats on gestation days (GDs) 6-15 at dose levels of 0, 30, 60, 125, or 250 mg/kg bw/day. The maternal LOAEL was 125 mg/kg bw/day, based on decreased body weight gain, and the maternal NOAEL is 60 mg/kg bw/day. At 250 mg/kg /day (the highest dose level tested), the pregnancy rate (as determined by positive Salewski staining) was significantly decreased, most likely due to preimplantation losses of entire litters. The tentative developmental LOAEL for α-terpinene in rats was 250 mg/kg bw/day, based on increased preimplantation loss and decreased fetal body weight. The tentative developmental NOAEL was 125 mg/kg bw/day. Due to the absence of individual data and the absence of litter data for the fetal morphological endpoints, the study is classified as **Acceptable/Non-guideline,** and it would not satisfy the *intent* of the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) with α-terpinene in the rat. However, because developmental toxicity was evident, this study could serve as a screening study and may provide useful supplemental information about the developmental toxicity of α-terpinene in rats.

Alpha-terpinene is one of the primary components of Tea Tree Oil. However, terpinen-4-ol is the component that is present at the highest percentage (approximately 38%, per MRID 48598704). Alpha-terpinene can be present in Tea Tree Oil at less than 10%, and together the five major components of Tea Tree Oil that were named by the registrant can comprise about 71.3%-73.49% of the material (MRIDs 48598701 and 48598702). Structural similarity is not a guarantee of similar biological activity. It is possible that one or more of the other components of Tea Tree Oil is of greater developmental toxicity or that the different components of Tea Tree

oil might interact in a way that increases their toxicity. For these reasons, α-terpinene is not a reasonable surrogate for Tea Tree Oil Technical. However, the fact that treatment with α-terpinene did result in developmental toxicity is reason for concern.

References

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Yogesha, B. 2011. Tea Tree Oil: 90-Day Repeated Dose Toxicity Study in Wistar Rats. Advinus Therapeutics Ltd., Dept. Safety Assessment, Toxicology, Bangalore, India. Laboratory Study Number: G7153. June 6, 2011. MRID 48598702.

APPENDIX

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; Non-guideline.

EPA REG NO.: 86182-E **DP BARCODE:** 396175

TEST MATERIAL (PURITY): α-Terpinene (89% a.i.)

SYNONYMS: 1-isopropyl-4-methyl-1,3-cyclohexadiene

CITATION: Araujo, 1., C. Souza, R. De-Carvalho, et al. (1996) Study of the embryofoetotoxicity of α-terpinene in the rat. Laboratory of Environmental Toxicology, Department of Biological Sciences, The National School for Public Health, Rio de Janeiro, Brazil. Laboratory report number not provided. MRID 48598704. Food and Chemical Toxicology, 34:477-482.

SPONSOR: Brazilian National Research Council. [Submitter is Biomor Israel Ltd., P.O. Box 81, Qatzrin, 12900 Israel.]

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 48598704) α-terpinene (89% a.i., batch/lot # not provided) was administered to 15-28 mated female Wistar rats/dose by gavage in corn oil at dose levels of 0, 30, 60, 125, or 250 mg/kg bw/day on gestation days (GDs) 6-15. Dams were sacrificed and necropsied on GD 21. All fetuses were weighed, sexed, and examined externally; approximately one-third of the fetuses in each litter were subjected to visceral examination (including collection of selected organ weights), and the remaining two-thirds were subjected to skeletal examination.

Maternal toxicity was seen at the 125- and 250-mg/kg dose levels. At 125 mg/kg bw, body weight gain was decreased during treatment (32% less than controls; $p<0.05$), and adjusted (for gravid uterine weight) cumulative body weight gain was 24% less than controls. The 250-mg/kg dams lost weight during GD 6-11 (-17.8 g vs. +13.6 g for controls) and had markedly decreased weight gain over the treatment interval (-95%; $p<0.05$) with adjusted (for gravid uterine weight) cumulative body weight gain 58% less than controls. The 250-mg/kg dams also had a decreased pregnancy rate (56 % vs. 86% for controls), which very likely was due to pre-implantation losses of complete litters in some of the animals.

The maternal LOAEL is 125 mg/kg bw/day, based on decreased body weight gain. The maternal NOAEL is 60 mg/kg bw/day.

Maternal treatment with α-terpinene did not result in increased numbers of resorptions or dead fetuses, but early (preimplantation) embryolethality cannot be definitively ruled out. Unequivocal growth impairment was evident at 250 mg/kg as decreased fetal weight (15% less than controls; $p<0.05$). Statistically significant increases in the incidence of fetuses with retarded ossification and fetuses with incomplete ossification of the supraoccipital bone of the skull and/or shorter ribs were seen at 125 and 250 mg/kg, and an increased incidence of fetuses with extra cervical rib(s)

was seen at 250 mg/kg. These differences cannot be definitely attributed to treatment due to the absence of litter data. Markedly decreased fetal thymus weights at 250 mg/kg bw/day (-30%; $p<0.05$) can be attributed to thymic atrophy related to maternal stress rather than a direct effect on the thymus. Very slight increases in fetal kidney weight were seen at 250 mg/kg bw/day; in the absence of correlated macroscopic observations, this is of undetermined significance.

The tentative developmental LOAEL for α-terpinene in rats is 250 mg/kg bw/day, based on increased preimplantation loss and decreased fetal body weight. The tentative developmental NOAEL is 125 mg/kg bw/day. However, if litter data for fetal ossification and alterations were available, these levels could be revised.

The developmental toxicity study in the rat is classified **Acceptable/Non-guideline.** Because the fetal incidences of the morphological findings were reported without litter data, this study would not satisfy the *intent* of the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) with α-terpinene in the rat. With provision of litter data and individual data, this study would meet the intent of the guideline requirement. Without these data, this study could serve as a screening study and may provide useful supplemental information.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** α-Terpinene

Description:	Not provided
Lot/batch #:	Not provided
Purity:	89% a.i.
Compound stability:	Not provided
CAS #of TGAI:	Not provided

2. **Vehicle and/or positive control:** The vehicle was corn oil (Mazola®; lot/batch number not provided). No positive control was used.

3. **Test animals:**

Species:	Rat	
Strain:	Wistar	
Age/weight at study initiation:	Not reported	
Source:	Oswaldo Cruz Central Animal House	
Housing:	Plastic cages with stainless-steel covers and with wood shavings as bedding	
Diet:	Nuvital® pelleted diet (Nuvilab Ltd., Curitiba, PR, Brazil), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	23±1EC
	Humidity:	approximately 70%
	Air changes:	not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Not reported	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** not available

2. **Mating:** The females were paired with sexually mature males of the same source and strain by caging the animals together (1 male:2 females) for 2 hours, 8:00-10:00 a.m. Mating was confirmed by the observation of spermatozoa in a vaginal smear, and the day on which the spermatozoa were observed was designated as gestation day (GD) 0.

3. **Animal assignment:** Animal assignment is given in Table 1. Mated females were assigned to dose groups in an unspecified manner.

TABLE 1. Animal assignment

Group	Control	Low-Dose	Low-Mid-Dose	High-Mid-Dose	High-Dose
Dose (mg/kg bw/day)	0	30	60	125	250
Number of Females	28	15	20	26	27

Data taken from p. 14, MRID 48598704.

4. **Dose selection rationale:** A dose selection rationale was not provided.

5. **Dosage preparation and analysis:** Test material-vehicle mixtures were prepared at an unspecified frequency, and the preparation method was not described in the study report. There was also no mention of evaluation of the stability of the test substance in the vehicle or concentration and homogeneity of the test mixtures. There was no data provided to indicate whether the mixing procedure was adequate or that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** Doses were administered once daily by gavage, on GDs 6 through 15. The control group received only corn oil at a dose level of 3.75 g/kg body weight. The dosing volumes administered to the treated groups were not reported, and it is unknown whether dosing was based on the body weight on a specific day of gestation or on the most recent body weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The mated females were weighed on (presumptive) GDs 0, 6-15, and 21 and sacrificed on GD 21 via decapitation under ethyl ether anesthesia. The animals were subjected to gross necropsy, and gravid uterine weight, corpora lutea counts, and the numbers of implantations, live and dead fetuses, and resorptions were recorded, with the Salewski staining technique used in determining the number of implantation sites. Food consumption and clinical signs observations were not mentioned or included in the report.

2. **Fetal evaluations:** Fetuses were weighed, sexed, examined externally, and fixed in 5% formalin solution. Approximately one-third of the fetuses in each litter (selected randomly) were subjected to visceral examination by a microsectioning technique (Sterz, 1977), and the hearts, lungs, thymuses, spleens, livers, and kidneys from these fetuses were weighed and

microdissected. The remaining fetuses were cleared with potassium hydroxide, stained with Alizarin Red S, and subsequently examined for skeletal abnormalities.

D. DATA ANALYSIS:

1. **Statistical analyses:** Data with a normal distribution were analyzed using one-way analysis of variance (ANOVA) followed by a two-sided Student's t-test if appropriate. Data that did not fit a normal distribution were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney U test, if appropriate. Proportions were analyzed using the chi-square test or Fisher's exact test. All tests used a significance level of $p<0.05$.

2. **Indices:** The study authors calculated and reported the percentage of implantations that were resorbed, and the percentage of implantations that resulted in live offspring.

3. **Historical control data:** Historical control data were not provided.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** Mortality and clinical signs data were not provided.

2. **Body weight:** Selected maternal body weight data are given in Table 2. Dose- and treatment-related effects on maternal body weight gain were seen at the upper two dose levels and were most marked during the first half of the treatment interval (GD 6-11). At 250 mg/kg/day, there was a resultant, small, but statistically significant decrease in mean absolute body weight on GD 21. Decreases in the adjusted (for gravid uterus) body gains of these groups indicate that these differences were due to maternal toxicity, at least in part.

TABLE 2. Mean (±SD) maternal body weights and body weight gains (g) [a]					
Gestation Day or Interval	Dose in mg/kg bw/day (# of Dams)				
	Control (24)	30 (14)	60 (18)	125 (25)	250 (15)
Absolute Body Weight					
GD 0	227±20	230±22	229±11	227±18	240±23
GD 21	348±29	347±39	357±23	341±28	324±29 * (-7) [b]
Gravid uterine weight	71.8±18.1	72.8±23.1	77.0±19.9	76.3±11.0	63.0±18.7
Body Weight Changes					
Pretreatment:					
GD 0-6	27.5±8.2	30.8±8.4	31.1±9.0	29.1±7.8	27.3±7.6
Treatment:					
GD 6-11	13.6±5.7	16.9±5.7	11.8±5.8	6.3±7.1 * (-54)	-17.8±12.9 *
GD 6-15	30.7±21.9	35.7±9.4	29.2±6.8	21.0±9.1 * (-32)	1.4±9.7 * (-95)
Post-treatment:					
GD 15-21	63.0±11.4	64.5±15.2	67.9±12.0	63.9±17.6	55.1±19.3
Cumulative GD 0-21	121.2±21.9	131.1±23.2	128.3±17.4	114.1±22.1	83.7±27.1 * (-31)
Adjusted GD 0-21 [c]	49.4±15.6	58.3±11.5	51.2±14.4	37.7±19.0 * (-24)	20.7±13.7 * (-58)

[a] Data taken from Table 1, p. 14, MRID 48598704.

[b] Numbers in parentheses equal percent different from control; calculated by reviewer.

[c] Adjusted for gravid uterine weight.

* Significantly different ($p<0.05$) from controls.

3. **Gross pathology:** The study authors stated that no abnormal gross findings were seen at necropsy.

4. **Cesarean section data:** Data collected at cesarean section are summarized in Table 3. There were no treatment-related effects on the percentage of implantations that were resorbed, the mean number of fetuses per dam, or fetal sex ratios, and the mean corpora lutea counts of the treated groups were similar to those of controls. At the highest dose level tested, the mean fetal weight and the pregnancy rate (as determined by positive Salewski staining) were significantly decreased.

   Because the individual data were not provided, the reviewer could not clarify or determine the cause of several reporting discrepancies, e.g. instances where the sum of total live fetuses and total resorptions exceeded the total number of implantations or where the sum of male and female fetuses did not equal the total number of fetuses. The reviewer was also unable to determine whether treatment resulted in increased percentages of dams with at least one resorption. Numbers of dead fetuses were not reported; because of the previously mentioned reporting discrepancies, the reviewer did not calculate these.

TABLE 3. Cesarean section observations [a] [Values given as Mean ± SD, where appropriate]					
Observation	Dose (mg/kg bw/day)				
	0	30	60	125	250
# Animals assigned (mated)	28	15	20	26	27
# Animals pregnant	24	14	18	25	15
Pregnancy rate (%)	86	93	90	96	56 *
# Animals that delivered early	0	0	1	0	0
Mean corpora lutea	12.5±3.0	12.9±2.1	12.6±2.5	12.9±1.8	12.1±2.9
Total # implantations/group	306	179	232	327	189
Mean implantations/dam	12.6±3.2	12.8±3.8	12.9±3.1	13.2±1.9	12.6±2.4
Total # of live fetuses/group	275	158	218	299	165
Mean live fetuses/dam	11.5±3.1	11.2±3.9	12.1±3.1	11.9±1.9	11.0±3.3
Total # of resorptions	37	27	15	27	24
Resorptions/implantations (%)	12.1	15.1	6.5	8.2	12.7
Sex ratio (% male) [b]	51.7	46.1	53.0	53.3	50.8
Mean fetal weight (g)	4.7±0.2	4.9±0.3	4.8±0.3	4.7±0.4	4.0±0.4 * (-15) [c]

[a] Data taken or derived from Tables 1 and 2, p. 14 and 15, respectively, MRID 48598704.

[b] Calculated by reviewer.

[c] Numbers in parentheses equal percent different from control; calculated by reviewer.

* Statistically different ($p < 0.05$) from controls.

B. DEVELOPMENTAL TOXICITY:

The total numbers of fetuses (and litters) evaluated in the control, 30-, 60-, 125-, and 250-mg/kg groups were 275 (24), 158 (14), 218 (18), 299 (25) and 165 (15), respectively. Morphological alterations and retarded ossification were reported as number (and/or percentage) of fetuses affected. No litter incidences or mean litter proportions were provided for these findings.

1. **External examination:** Selected findings from the external examinations are given in Table 4a. Due to the lack of a dose response, none of these findings were considered treatment-related.

2. **Visceral examination:** Selected findings from the visceral examinations are given in Table 4b, and selected absolute organ weight data are given in Table 4c. Treatment-related organ weight differences (decreased thymus weight and slightly increased kidney weights) were seen in fetuses from dams treated at 250 mg/kg. Other statistically significant differences in mean absolute organ weights were seen (such as increased absolute kidney weights in fetuses from dams treated at 60 and 125 mg/kg), but all were small in magnitude and/or attributable to differences in fetal body weight. All visceral anomalies in fetuses from treated dams were seen at single incidences; therefore none were considered treatment-related.

3. **Skeletal examination:** Selected findings from the skeletal examinations are given in Table 4d. At 60, 125, and 250 mg/kg, there were dose-related statistically significant increases in the incidence of fetuses with retarded ossification and the incidence of fetuses with the skeletal anomaly irregularly shaped os squamosum. Increased incidences of fetuses with incomplete ossification of the supraoccipital bone of the skull and/or shorter ribs were seen at 125 and 250 mg/kg, and an increased incidence of fetuses with extra cervical rib(s) was seen at 250 mg/kg.

TABLE 4a. External anomalies [a] [Number (and %) fetuses affected]					
Observations	Dose in mg/kg bw/day				
	Control	30	60	125	250
Number examined	275	158	218	299	165
Hematoma	10 (3.6%)	7 (4.4%)	7 (3.2%)	16 (5.3%)	13 (7.9%)
Bent tail	1 (0.4%)	0	2 (0.9%)	6 (2.0%)	4 (2.4%)
Kinky tail	3 (1.1%)	10 (6.3%) *	3 (1.4%)	6 (2.0%)	5 (3.0%)
Irregular positioning of fore paws	0	1 (0.6%)	0	4 (1.3%)	0
Irregular positioning of hind paws	2 (0.7%)	2 (1.3%)	1 (0.4%)	3 (1.0%)	2 (1.2%)

[a] Data taken from Table 4, p. 16, MRID 48598704.
* Significantly different ($p < 0.05$) from controls.

TABLE 4b. Visceral anomalies [a] [Number (and %) fetuses affected]					
Observations	Dose in mg/kg bw/day				
	Control	30	60	125	250
Number examined	86	49	67	92	51
Ectopic testes	3 (3.5%)	0	1 (1.5%)	1 (1.1%)	0
Ectopic spleen	1 (1.2%)	0	0	0	0
Small heart	0	1 (2.0%)	0	0	0
Small liver	1 (1.2%)	0	0	0	0
Small adrenal gland	0	0	1 (1.5%)	0	0
Thick ureter	0	0	0	0	1 (2.0%)

[a] Data taken from Table 4, p. 16, MRID 48598704.

TABLE 4c. Selected absolute fetal organ weights [a] [mg]					
Observations	Dose in mg/kg bw/day				
	Control	30	60	125	250
Number examined	86	49	67	92	51
Fetal body weight	4.9±0.5	5.3±0.5	5.3±0.4	5.2±0.5	4.2±0.5 * (-14) [b]
Organ weights:					
Right kidney	10.8±2.0	11.1±1.7	12.2±1.7 * (13)	12.1±2.4 * (12)	11.8±2.0 * (9)
Left kidney	10.4±2.2	10.4±1.6	11.4±1.7 * (10)	11.1±2.0 * (7)	12.0±2.0 * (15)
Thymus	7.6±1.1	7.4±1.5	8.0±1.4	7.5±1.6	5.3±1.7 * (-30)

[a] Data taken from Table 5, p. 16, MRID 48598704.
* Significantly different ($p < 0.05$) from controls.

TABLE 4d. Skeletal observations [a] [% fetuses affected]					
Observations	Dose in mg/kg bw/day				
	Control	30	60	125	250
Number examined	189	109	151	207	114
Anomalies					
Total affected fetuses	19.6%	27.5%	33.1% *	61.3% *	89.5% *
Skull - irregularly shaped os squamosum	4.8%	6.4%	13.2% *	24.6% *	35.1% *
Skull - incomplete ossification of os supraoccipitale	0.5%	0.9%	2.6%	12.6% *	36.0% *
Ribs - Shorter	5.8%	6.4%	6.0%	19.8% *	50.0% *
Ribs - Extra cervical rib(s)	0.5%	0.9%	1.3%	1.0%	7.0% *
Forelimb anomaly or anomalies [b]	2.6%	5.5%	6.6%	17.9% *	6.1%
Retarded ossification					
Total affected fetuses	11.1%	14.7%	53.0% *	73.4% *	88.6% *
Skull bones	0.5%	4.6% *	2.6%	2.9% *	16.6% *
Vertebral column	1.6%	0.9%	22.5% *	34.8% *	21.0% *
Sternum	11.6%	5.5% *	45.0% *	70.0% *	87.7% *
Ribs	0	0	6.0% *	13.5% *	6.1% *
Forelimbs	1.6%	1.8%	13.2% *	9.2% *	9.6% *
Hind limbs	4.8%	9.2%	37.7% *	37.2% *	47.4% *

[a] Data taken from Tables 3 and 6, pp. 15 and 17, respectively, MRID 48598704.

[b] Includes irregular shape and/or "bone hole" of the os processus deltoid and/or irregular position of the forelimb(s).

* Significantly different ($p < 0.05$) from controls.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that maternal toxicity was evident at 125 and 250 mg/kg bw/day as decreased body weight gain and/or body weight loss during gestation. According to the study authors, developmental toxicity was evident at 60, 125, and 250 mg/kg as delayed ossification and minor skeletal anomalies, with additional effects seen at 250 mg/kg, including decreased fetal weight, decreased thymus weight, and increased kidney weight. The study authors stated that the decreased pregnancy rate of the 250-mg/kg females may have been due to impaired implantation and concluded that the treatment-related, whole litter, peri-implantation losses were probably a maternally mediated effect, associated with the marked toxicity seen at this dose level. The study authors also concluded that no adverse effects on embryofetal development were seen at the lowest dose level and set a NOAEL of 30 mg/kg bw/day for the developing fetus.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** The reviewer agrees that evidence of maternal toxicity was seen at the 125- and 250-mg/kg dose levels. The decreased pregnancy rate of the 250-mg/kg females very likely was due to pre-implantation losses of complete litters in some of the animals. As stated by the study authors, this probably was related to maternal stress. Given that treatment was initiated around the time of implantation, other potential mechanisms would include

uterine toxicity, an effect on the process of implantation, or embryolethality resulting from direct toxicity to the conceptus.

The maternal LOAEL is 125 mg/kg bw/day, based on decreased body weight gain. The maternal NOAEL is 60 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Maternal treatment with α-terpinene did not result in increased numbers of resorptions or dead fetuses. Occurrence of treatment-related early (preimplantation) embryolethality cannot be definitively ruled out.

   b. **Altered growth:** Unequivocal growth impairment was evident at 250 mg/kg as decreased fetal weight, possibly with concurrent delayed ossification. In disagreement with the study authors, the reviewer considers the statistically significant increases in the incidence of fetuses with retarded ossification in the 60- and 125-mg/kg groups to be of undetermined significance in the absence of litter data.

   c. **Structural alterations:** The reviewer also considers the increased incidences of fetuses with incomplete ossification of the supraoccipital bone of the skull and/or shorter ribs (at 125 and 250 mg/kg) and an increased incidence of fetuses with extra cervical rib(s) (at 250 mg/kg) to be of undetermined significance in the absence of litter data.

   d. **Specific organ toxicity:** The reviewer agrees that the organ weights of the thymus and kidney were affected by maternal treatment at the 250-mg/kg dose level. The decreased thymus weight was most likely due to atrophy as the result of maternal stress rather than a direct effect on the thymus. In the absence of correlated gross structural changes, the increased kidney weight is of undetermined significance and potentially adverse.

   In developmental toxicity studies, the litter is considered the experimental unit. In interpreting the results of the external, visceral, and skeletal examinations, the number of affected litters should be considered first, and both the number of fetuses and the number of litters are important in making a final conclusion. Because the fetal incidences of the morphological findings were reported without litter data, the increased incidences of fetuses with delayed ossification and/or minor skeletal anomalies cannot be definitely attributed to treatment, and these endpoints, therefore, cannot be used as the basis for setting a LOAEL. **Therefore, the tentative developmental LOAEL for α-terpinene in rats is 250 mg/kg bw/day, based on increased preimplantation loss and decreased fetal body weight, and the tentative developmental NOAEL is 125 mg/kg bw/day. However, if litter data for fetal ossification and alterations were available, these levels could be revised.**

C. **STUDY DEFICIENCIES:** Because the fetal incidences of the morphological findings were reported without litter data, this study would not satisfy the *intent* of the guideline requirement for a developmental toxicity study (OCSPP 870.3700; OECD 414) with α-terpinene in the rat.

This study also deviated from OCSPP 870.3700 requirements with respect to the following:

- Individual data were not provided.

- The test material was not fully characterized.
- It is unknown whether sibling matings were avoided.
- Dead fetuses were not reported. If there were none, this should have been stated.
- Resorptions were not characterized and reported as early/late.
- Fetal body weight data were not reported separately by sex.

DATA EVALUATION RECORD

Timorex Gold/Tea Tree Oil Screening Level Dietary Risk Assessment

STUDY TYPE: Risk Assessment
(Nonguideline)

MRID 48602801

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task 4, Work Order No. 11-024

Primary Reviewer:
Dennis M. Opresko, Ph.D.

Signature: 
Date: JAN 0 4 2012

Secondary Reviewers:
Tom Marshall, Ph.D., D.A.B.T.

Signature: Tom Marshall, AE
Date: JAN 0 4 2012

Robert H. Ross, M.S. Program Manager

Signature: Robert H. Ross
Date: JAN 0 4 2012

Quality Assurance:
Angie Edmonds, B.S.

Signature: Angie Edmonds
Date: JAN 0 4 2012

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Summitec Corp. for the U.S. Environmental Protection Agency under Contract No.EP-W-11-014

THIS DATA EVALUATION RECORD HAS BEEN ANNOTATED WITH PEER REVIEW COMMENTS FROM MICROBIAL AND BIOCHEMICAL EVALUATION SECTION (MBES)/PMRA (MARCH 27, 2012)

DATA EVALUATION RECORD

Timorex Gold/Tea Tree Oil Screening Level Dietary Risk Assessment

STUDY TYPE: Risk Assessment
(Nonguideline)

MRID 48602801

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
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One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task 4, Work Order No. 11-024

Primary Reviewer:
Dennis M. Opresko, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Tom Marshall, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S. Program Manager Signature: _____
Date: _____

Quality Assurance:
Angie Edmonds, B.S. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales 1/24/13 /s/**
STUDY TYPE:	Risk Assessment (Nonguideline)
MRID NO:	48602801
DP BARCODE:	396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	Timorex Gold – Tea Tree Oil
STUDY NO:	None
SPONSOR:	Biomor Israel Ltd., PO Box 81, Qatzrin, 12900 Israel
TESTING FACILITY:	Not applicable
TITLE OF REPORT:	Tea Tree Oil Screening Level Dietary Risk Assessment
AUTHOR:	B.E. Mileson
STUDY COMPLETED:	September 12, 2011
CONFIDENTIALITY CLAIMS:	No claim of confidentiality
GOOD LABORATORY PRACTICE:	"This study does not meet the requirements of 40 CFR Part 160. A Quality Assurance Unit was not in place. A Study Director was not assigned."
STUDY SUMMARY:	A screening level dietary risk assessment was conducted using: 1) the results of two vegetable residue trials (one on sweet peppers and the other on tomatoes); 2) food consumption data by age group from the U.S. EPA Exposure Factors Handbook; and 3) the results of a 90-day oral exposure study in rats from which an oral RfD was estimated. Forty-eight hours after application, residue levels on both crops diminished to the LOD of 0.05 mg/kg. The dietary exposure assessment used an average residue level of 0.1 mg/kg and the 99th percentile age-specific food consumption values for fruit, vegetables and grains to estimate daily intakes. The highest estimated intake of 0.0146 mg/kg bw/day was for children < 1 year old. The 90-day rat oral toxicity study resulted in a NOEL of 30 mg/kg bw/day. Application of a total Uncertainty Factor of 100 gave an estimated RfD of 0.3 mg/kg/day. The study author concluded that the maximum potential exposure of 0.0146 mg/kg/day was well below the estimated RfD of 0.3 mg/kg/day and below any level of potential concern.
CLASSIFICATION:	**EPA Reviewer Note: The information presented below**

is considered supplemental in the dietary risk assessment. At this time, this assessment will not be used in the Agency's risk assessment for tea tree oil. Due to uncertainties identified regarding residues and toxicity of the test substance, the Agency cannot make a safety finding to support a tolerance exemption at this time.

Contractor Review: Unacceptable/upgradeable. Critical to the dietary risk assessment is the calculation of the estimated oral RfD which is based on a composite Uncertainty Factor of 100. It was not stated in the study report which individual or combination of uncertainty factors were applied. The approach appears reasonable and protective for calculating risks to select age groups under the assumption that the exposure would be subchronic in duration. A larger composite UF (one that would include a subchronic to chronic extrapolation) would be necessary to estimate an oral RfD for chronic exposure scenarios, in which case an estimate would be needed of the daily intake over a lifespan. The registrant needs to address this issue.

The dietary risk assessment compared exposures resulting from residues of the primary active ingredients in Timorex Gold to toxicity data for Tea Tree Oil technical. Consequently, the potential dietary risks associated with the non-active ingredients of Timorex Gold (76.2% of the total) were not discussed. The registrant needs to address this issue.

Test Material

Timorex Gold (23.8% Tea Tree Oil); and a 66% active ingredient preparation (it was not explained how the 66% a.i. test material was prepared and whether or not it contained the same types of non-a.i. ingredients as that of Timorex Gold).

Background

Timorex Gold is being registered for use on horticultural and agricultural plants to prevent and control powdery mildew, downy mildew, Early and late blight, Botrytis, rust, Apple scab and black sigatoka. Timorex Gold is recommended for use on edible fruits, vegetables and grains, as well as herbs and vine crops. The registrant has conducted a screening level dietary risk assessment using the results of two vegetable residue trials; one on sweet peppers and the other on tomatoes.

Methods and Materials

Residue information from two field trials conducted on sweet peppers and tomatoes, and dietary intake values for fruits vegetables and grains (from U.S. EPA Exposure Factors Handbook) were used to estimate daily oral exposures. The resulting exposure estimates were then used with an estimated oral RfD, derived from a 90-day oral toxicity study in rats, in a screening level dietary risk assessment.

Field Trials: Timorex Gold was applied to sweet peppers and tomatoes at six different application rates at two different sites in Spain and Holland (Chadwick, 2008; MRID 47730522). Four plots at each site were treated with different concentrations of the 23.8% a.i. end use product (1190, 1785, 2380, and 3570 g a.i./hectare) and two plots were treated with a 66% a.i. preparation at higher application rates (6600 and 13200 g a.i./hectare). The maximum label application rate, as calculated by the study author, is 1788 g a.i. per hectare. The sweet peppers and tomatoes were tested for tree oil residues immediately after the applied product had dried and at 6, 24 and 48 hr after application. A maximum residue concentration (terpinen-4-ol) of 0.36 mg/kg was detected immediately after the product was applied (and dried) in the plot with the highest application rate. The residue levels decreased over time such that by 48 hr the residues were below the Limit of Detection of 0.05 mg/kg. Based on the results of the study, the label recommended pre-harvest interval (PHI) was changed to 48 hr to ensure adequate dissipation and degradation of the product. In the screening level dietary risk assessment, twice the Limit of Detection (0.1 mg/kg) was used as the residue level on all potential crops to be treated with Timorex Gold (fruits, vegetable and grains).

Exposure Assessment (Dietary Intakes): The exposure assessment assumes use of Timorex Gold on all crops for which it is approved (fruits, vegetables and grains). Daily intakes of these food categories by age group were obtained from the U.S. EPA Exposure Factors Handbook. The daily intakes of tea tree oil from these foods, individually and in total were calculated using a conservative assumption of a residue level of 0.1 mg/kg and the 99th percentile fruit, vegetable, and grains daily intakes. Results are shown in the following table.

Table 1. Screening Level Exposure Assessment - Tea Tree Oil from Timorex Gold.

Dietary Exposure Assessment			
Tea Tree Oil			
Residue = 2X the LOD; assume 48 hour Pre Harvest Interval			
EFH 89-91; 99th Percentile	**Age <1**		
Food Group	M&F <1 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	29.61	0.1	0.002961
Fruits (Total)	88.42	0.1	0.008842
Grain Products (Total)	27.61	0.1	0.002761

		Sum:	0.014564
EFH 89-91; 99th Percentile	**Age 1 - 2**		
Food Group	M&F 1-2 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	21.24	0.1	0.002124
Fruits (Total)	52.27	0.1	0.005227
Grain Products (Total)	28.22	0.1	0.002822
		Sum:	0.010173
EFH 89-91; 99th Percentile	**Age 3-5**		
Food Group	M&F 3-5 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	25.09	0.1	0.002509
Fruits (Total)	32.83	0.1	0.003283
Grain Products (Total)	23.87	0.1	0.002387
		Sum:	0.008179

EFH 89-91; 99th Percentile	**Age 6-11**		
Food Group	M&F 3-11 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	18.39	0.1	0.001839
Fruits (Total)	21.53	0.1	0.002153
Grain Products (Total)	21.4	0.1	0.00214
		Sum:	0.006132
EFH 89-91; 99th Percentile	**Age 12-19**		
Food Group	M&F 12-19 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	12.26	0.1	0.001226
Fruits (Total)	11.8	0.1	0.00118
Grain Products (Total)	10.92	0.1	0.001092
		Sum:	0.003498
EFH 89-91; 99th Percentile	**Age 20-39**		
Food Group	M&F 20-39 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	10.69	0.1	0.001069
Fruits (Total)	10.26	0.1	0.001026
Grain Products (Total)	9.57	0.1	0.000957
		Sum:	0.003052
EFH 89-91; 99th Percentile	**Age 40-69**		
Food Group	M&F 40-69 Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	10.91	0.1	0.001091
Fruits (Total)	10.52	0.1	0.001052
Grain Products (Total)	8.4	0.1	0.00084
		Sum:	0.002983
EFH 89-91; 99th Percentile	**Age 70+**		
Food Group	M&F 70+ Age (g/kg bw/day)	Est. Residue (mg/kg)	Exposure (mg/kg bw/day)
Vegetables (Total)	11.96	0.1	0.001196
Fruits (Total)	11.89	0.1	0.001189
Grain Products (Total)	10.47	0.1	0.001047
		Sum:	0.003432

Toxicity Data and Calculation of Estimated Oral RfD: A 90-day oral toxicity study conducted in rats (Yogesha, 2011) resulted in a NOEL of 30 mg/kg/day. In addition, a surrogate developmental toxicity study also resulted in a NOEL of 30 mg/kg/day (Araujo et al., 1996). The NOEL of 30 mg/kg/day was used by the study author to estimate an oral RfD of 0.3 mg/kg/day using a composite Uncertainty Factor of 100 (specific components of the Uncertainty Factor were not identified).

Results

As shown in Table 1, the maximum potential exposure to residues of Timorex Gold on fruit, vegetables and grains combined was 0.0146 mg/kg/day for the age group of < 1 year old. The estimated RfD was 0.3 mg/kg/day, about 20 times greater than the maximum theoretical exposure.

Study Author's Conclusions

The study author concluded that the maximum theoretical exposure was below any level of potential concern.

Reviewer's Conclusion

The approach used by the study author to calculate the maximum potential exposure to Timorex Gold residues on fruit, vegetable and grain crops was a reasonable and health protective one. The conservative assumptions were made that all crops consumed would have been treated with Timorex Gold, that the residue levels would be two times the Limit of Detection, and that daily intake of fruits, vegetables and grains would be equivalent to the 99th percentile values. There is no EPA-approved oral RfD for tea tree oil; therefore, the study author estimated one from the available animal toxicity studies on Tea Tree Oil technical (MRIDs 48598702 and 48598704, review pending). These studies resulted in a subchronic NOEL of 30 mg/kg. The study author estimated the oral RfD of 0.3 mg/kg/day from this NOEL by applying an Uncertainty Factor of 100. The study author did not specifically identity the individual uncertainty factors used in this derivation; however, it would appear that a composite UF of 100 (10 for extrapolating from animals to humans and 10 for sensitive subpopulations) would be appropriate for calculating risk to select age groups as was done in MRID 48602801. It could, however, be argued that a composite UF of 1000 (an additional UF of 10 for extrapolating from a subchronic to chronic exposure) would be necessary for calculating risk over an entire life span, in which case the resulting estimated oral RfD of 0.03 mg/kg/day would have to be compared to the estimated average daily intake of tea tree oil over 70 years. The reviewer recommends that this additional calculation be made.

NOTE #1: Although the exposure assessment included daily intake of fruits, vegetables and grains, it does not appear that liquids derived from these food products, such as fruit and vegetable juices and wines and other products, were included in the assessment.

NOTE #2. The dietary risk assessment compared exposures resulting from residues of the primary active ingredients in Timorex Gold to toxicity data for Tea Tree Oil technical.

Consequently, the potential dietary risks associated with the non-active ingredients of Timorex Gold (76.2% of the total) were not addressed.

REFERENCES

NOTE: Some of the reports cited in this review are data submissions to the Agency that may not yet have undergone review by the EPA; therefore, the acceptability of the data cannot in all cases be verified.

Araujo IB, Souza CAM, De-Carvalho RR, Kuriyama, SN, Rodrigues, RP, Vollmer RS, Alves EN, Paumgartten FJR. 1996. Study of the embryofoetotoxictity of α-terpinene in the rat. Food and Chemical Toxicology, 34;477-482.

Chadwick G, 2008. Final report on project AF/12583/BB. OPPTS Guideline Number 860.1300. Agrisearch UK Ltd. Project No. AF/12583/BB. MRID No. 47730522

U.S. EPA, 1997. Exposure Factors Handbook. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development. http://www.epa.gov/ncea/efh/pdfs/efh-front-gloss.pdf

Yogesha BN, 2011. Tea Tree Oil: 90-day repeated dose toxicity study in Wistar rats. Advinus Therapeutics Limited, Study No G7153, Bangalore 560 058, India.

DATA EVALUATION RECORD

Timorex Gold

STUDY TYPE: Nature of the Residue (OCSSP 860.1300)

MRID 48275007

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task 4, Work Order No. 11-024

Primary Reviewer:
Dennis M. Opresko, Ph.D.

Signature: _____
Date: JAN 0 4 2012

Secondary Reviewers:
Eric Lewis, M.S.

Signature: Eric B. Lewis
Date: JAN 0 4 2012

Robert H. Ross, M.S. Program Manager

Signature: _____
Date: JAN 0 4 2012

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: Jennifer Goldberg
Date: JAN 0 4 2012

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Summitec Corp. for the U.S. Environmental Protection Agency under Contract No. EP-W-11-014

NO PEER REVIEW COMMENTS FROM MICROBIAL AND BIOCHEMICAL EVALUATION SECTION (MBES)/PMRA ON THIS DATA EVALUATION RECORD (MARCH 27, 2012)

DATA EVALUATION RECORD

Timorex Gold

STUDY TYPE: Nature of the Residue (OCSSP 860.1300)

MRID 48275007

Prepared for
Biopesticides and Pollution Prevention Division
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One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

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Robert H. Ross, M.S. Program Manager

Signature: _____
Date: _____

Quality Assurance:
Jennifer Goldberg, B.S.

Signature: _____
Date: _____

Disclaimer

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DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales 1/26/13 /s/**
STUDY TYPE:	Nature of the Residue (OCSSP 860.1300)
MRID NO:	48275007
DP BARCODE:	396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	Timorex Gold
STUDY NO:	Lab Project ID, S08-02382
SPONSOR:	Biomor Israel Ltd., PO Box 81, Qatzrin, 12900 Israel
TESTING FACILITY:	Eurofins Agroscience Services, Slade Lane, Wilson, Melbourne, Derbyshire DE73 8AG, UK
TITLE OF REPORT:	Determination of residues of Tea Tree Oil after a single application, at harvest, of BM-608 in sweet peppers, tomatoes and cucumbers (indoor) at six sites in Europe 2008
AUTHOR:	C. Ellis
STUDY COMPLETED:	April 21, 2009
CONFIDENTIALITY CLAIMS:	No claim of confidentiality
GOOD LABORATORY PRACTICE:	"This study does not meet the requirements of 40 CFR Part 160. This study was conducted in accordance with the Good Laboratory Practice Regulations of the OECD [OECD series number 1 and 13, ENV/MC/CHEM (98) 17, and ENV/JM/MONO (2002) 9]."
STUDY SUMMARY:	Six indoor residue trials were conducted with Timorex Gold (BM 608): one on tomatoes in Italy; one on sweet peppers in Greece; and four on cucumbers in southern France, Germany, the Netherlands, and the United Kingdom. At each site BM-608 (23.8% w/w tea tree oil) was diluted with water and applied at two concentrations; 0.5 L product/hl (119 g a.i./hl) and 1.0 L product/hl (238 g a.i./hl). Control plots were untreated. At all sites samples were taken for analysis by hand immediately after the application had dried and at 6 hr; in addition, at the sites in France and Germany samples were also taken at 12 hr after application, and at the site in the UK samples were taken at

24 and 48 hr after application. Crop specimens were analysed for residues of terpinen-4-ol, gamma-terpinene and 1,8-cineole using Agrisearch Method "Terpinen-4-ol, Gamma-Terpinene and 1,8-cineole/Crops/JAC/06/1". Final determination was performed by gas chromatography with mass spectrometry detection (GC-MSD). The analytical method was validated during analysis. The limit of quantification of the method was set at 0.05 mg/kg. Untreated and treated specimens received a single assay. No residues above the limit of quantification were found in any of the untreated specimens. At all sites for all three crops and for both test concentrations tea tree oil residues were below the limit of quantification of 0.05 mg/kg (for those chemicals analyzed).

CLASSIFICATION: **Acceptable.**

Test Material

BM 608 (a.i. 23.8 w/w tea tree oil, purity as specified by the sponsor – a Certificate of Analysis was not included in the study report; identified as Timorex Gold in the cover letter dated Oct. 6, 2010); batch numbers 6162 and 6114; clear palm yellow liquid with expiration dates of July 2009 and November, 2009, respectively. After receipt at the test facility, the test material was stored at ca. 5-30°C. The percent composition of α-terpinene; 1,8-cineole; g-terpinene; p-cymene; and terpinen-4-ol in the test product was not specified.

Background

In October of 2010, the registrant, Biomor Israel, Ltd., submitted residue data in support of the pending registration of Tea Tree Oil Technical (EPA Reg. No. 86182-E; 100% a.i.) and the end use product Timorex Gold (EPA Reg. No. 86182-R; 23.8% a.i.). The registrant stated that the data submitted were not to address any deficiencies in the submission, but to help create a complete data set on the active ingredient and proposed end use product.

Methods and Materials

Six residue trials were conducted; one on tomatoes in Italy (S08-02382-01 (1304)), one on sweet pepper in Greece (S08-02382-02; 1305)) and four on cucumbers in southern France (S08-02382-04; 1307), Germany (S08-02382-05; 1308), The Netherlands (S08-02382-03; 1306), and the UK (S08-02382-06; 1309). At each site, one application of BM-608 (23.8 w/w tea tree oil), diluted with water immediately prior to application, was applied to two plots, Plot 2 at 0.5 L product/hl (119 g a.i./hl) and Plot 3 at 1.0 L product/hl (238 g a.i./hl). Control plots were untreated. In trials S08-02382-01, 02 and 03 (1304, 1305 and 1306), samples of each crop from the untreated and treated plots were taken by hand immediately after the application had dried, and 6 hours after application. In trials S08-02382-04 and 05 (1307 and 1308), samples were taken by hand immediately after the application had dried, and 6 and 12 hours after application. In trial S08-02382-06 (1309), samples were taken by hand immediately after the application had dried, and 6,

24 and 48 hours after application. Samples were frozen 1-7 hr after collection and maintained frozen until analysis.

Crop specimens were analyzed for residues of terpinen-4-ol, gamma-terpinene and 1,8-cineole using Agrisearch Method "Terpinen-4-ol, Gamma-Terpinene and 1,8-Cineole/Crops/JAC/06/1." The method involves extraction in ethyl acetate in the presence of anhydrous sodium sulphate. Extracts were then centrifuged. Final determination was performed by gas chromatography with mass spectrometry detection (GC-MSD). The analytical method was validated during analysis. The limit of quantification of the method was set at 0.05 mg/kg. Recovery of terpinen-4-ol, gamma-terpinene and 1,8-cineole in sub-samples of each crop matrix spiked at concentrations of 0.05 and 0.5 mg/kg were determined. Untreated and treated specimens were assayed once.

Field Trial Protocols: Site characterization and product application details for the tea tree oil residue trial conducted on tomatoes in Italy are shown in Tables 1 and 2, respectively.

Table 1. Trial No. S08-02382-01 (1304) – Site Description

Location	
Postcode, town, county/federal state/département, country	Fondi, Italy, 04022
Location reference/ GPS coordinates	41.313202N, 13.338369E
Crop	
Test system	Protected Tomato (*Lycopersicon esculentum*) - Bayer code ref. LYPES
Cultivar/Variety	Saddaya
Planting or seeding date	01 Apr 2008
Plot Details	
Plot size width x length, other	4 m x 5 m
Closest distance between control and treated plot(s)	10 m
Closest distance between treated plots	3 m
Distance between rows	35cm
plants per plot	114
TRV parameters	8040 m^3/ha
Slope	Flat

Table 2. Trial No. S08-02382-01 (1304) – Application Details

TREATMENT NO.	2	3
Application code	A1	A1
Application date	17 Jul 2008	17 Jul 2008
Application equipment	Knapsack sprayer	Knapsack sprayer
Nozzle type	Cone	Cone
Nozzle size	nr	nr
Spray pressure (bar)	nr	nr
Type of application	Directed application	Directed application
Application volume actual (L/ha)	1440	1450
g ai/hl applied[a]	119	238
L product /hl applied[a]	0.5	1.0
Crop growth stage (BBCH)	75	75
Stand height of crop (m)	2.1	2.1
Target conditions	dry	dry
Crop ground cover (%)	50	50
Actual air temperature (°C)	30.0	30.0
Wind speed (m/s)	na	na
Actual relative air humidity (%)	93	93
Cloud cover (%)	15	15
Wetness of soil - 2-5 cm	Damp	Damp
Temperature of soil - 2-10 cm (°C)	24.0	24.0

[a] based on nominal content

Site characterization and product application details for the tea tree oil residue trial conducted on sweet peppers in Greece are shown in Tables 3 and 4, respectively.

Table 3. Trial No. S08-02382-02 (1305) – Site Description

Location	
Postcode, town, county/federal state/département, country	Nea Magnisia, Ionia, Greece, GR-57008
Location reference/ GPS coordinates	40.69187 N, 22.85051 E
Crop	
Test system	Protected Sweet Pepper (*Capsicum annuum*) - Bayer code ref. CPSAN
Cultivar/Variety	Gemisti
Planting or seeding date	05 May 2008
Plot Details	
Plot size width x length, other	3.2 m x 10 m
Closest distance between control and treated plot(s)	10 m
Closest distance between treated plots	10 m
Distance between rows	80 cm
plants per plot	100
TRV parameters	3093.75 m^3/ha
Slope	Flat

Table 4. Trial No. S08-02382-02 (1305) – Application Details

TREATMENT NO.	2	3
Application code	A1	A1
Application date	24 Jul 2008	24 Jul 2008
Application equipment	Knapsack sprayer	Knapsack sprayer
Nozzle type	Cone	Cone
Nozzle size	nr	nr
Spray pressure (bar)	3	3
Type of application	Directed application	Directed application
Application volume actual (L/ha)	1006	978
g ai/hl applied[a]	119	238
L product /hl applied[a]	0.5	1.0
Crop growth stage (BBCH)	87-89	87-89
Stand height of crop (m)	0.7	0.7
Target conditions	dry	dry
Crop ground cover (%)	50	50
Actual air temperature (°C)	25.7	26.3
Wind speed (m/s)	na	na
Actual relative air humidity (%)	48	56
Cloud cover (%)	10	10
Wetness of soil - 2-5 cm	Damp	Damp
Temperature of soil - 2-5 cm (°C)	19.0	19.0

[a] based on nominal content

Site characterization and product application details for the tea tree oil residue trial conducted on cucumbers in The Netherlands are shown in Tables 5 and 6, respectively.

Table 5. Trial No. S08-02382-03 (1306) – Site Description

Location	
Postcode, town, county/federal state/département, country	Steenbergerveld 11, 6851 EA Huissen (Gelderland), The Netherlands
Location reference/ GPS coordinates	n/r
Crop	
Test system	Protected Cucumber (*Cucumis sativus*) - Bayer code ref. CUMSA
Cultivar/Variety	Shella
Planting or seeding date	05 Aug 2008
Plot Details	
Plot size width x length, other	5.2 m x 10 m
Closest distance between control and treated plot(s)	n/r
Closest distance between treated plots	n/r
Distance between rows	130 cm
Rows per plot	4
TRV parameters	n/r
Slope	Flat
Soil Classification	
Soil type / pH (from appearance and texture)	Rockwool / pH 6.5

Table 6. Trial No. S08-02382-03 (1306) – Application Details

TREATMENT NO.	2	3
Application code	A1	A1
Application date	14 Oct 2008	14 Oct 2008
Application equipment	Vertical plot sprayer	Vertical plot sprayer
Nozzle type	Flat fan	Flat fan
Nozzle size	80015 VK	80015 VK
Spray pressure (bar)	4	4
Type of application	overall	overall
Application volume actual (L/ha)	1042	1056
g ai/hl applied	124	251
L product /hl applied[a]	0.6	1.1
Crop growth stage (BBCH)	78-79	78-79
Stand height of crop (m)	n/r	n/r
Target conditions	dry	dry
Crop ground cover (%)	n/r	n/r
Actual air temperature (°C)	21.3	21.3
Wind speed (m/s)	0	0
Actual relative air humidity (%)	78.5	78.5
Cloud cover (%)	100	100
Wetness of soil - 2-5 cm	n/r	n/r
Temperature of soil - 10 cm (°C)	18.0	18.0

Site characterization and product application details for the tea tree oil residue trial conducted on cucumbers in France are shown in Tables 7 and 8, respectively.

Table 7. Trial No. S08-02382-04 (1307) – Site Description

Location	
Postcode, town, county/federal state/département, country	Alenya, Languedoc Roussillon, France, 66200
Location reference/ GPS coordinates	42° 37' 471" N, 02° 59' 031" E
Crop	
Test system	Protected Cucumber (*Cucumis sativus*) - Bayer code ref. CUMSA
Cultivar/Variety	Airbus
Planting or seeding date	15 Apr 2008
Plot Details	
Plot size width x length, other	4 m x 10 m
Closest distance between control and treated plot(s)	18 m
Closest distance between treated plots	0 m
Distance between rows	1.0cm
plants per plot	133
TRV parameters	13804 m^3/ha
Slope	Flat

Table 8.Trial No. S08-02382-04 (1307) – Application Details

TREATMENT NO.	2	3
Application code	A1	A1
Application date	27 Jun 2008	27 Jun 2008
Application equipment	Knapsack sprayer	Knapsack sprayer
Nozzle type	Cone	Cone
Nozzle size	nr	nr
Spray pressure (bar)	13	nr
Type of application	Directed application	Directed application
Application volume actual (L/ha)	968	1005
g ai/hl applied[a]	119	238
L product /hl applied[a]	0.5	1.0
Crop growth stage (BBCH)	89	89
Stand height of crop (m)	2.0	2.0
Target conditions	Dry	Dry
Crop ground cover (%)	40	40
Actual air temperature (°C)	24.1	24.1
Wind speed (m/s)	na	na
Actual relative air humidity (%)	47	47
Cloud cover (%)	10	10
Wetness of soil - 2-5 cm	Dry	Dry
Temperature of soil - 2-5 cm (°C)	26.0	26.0

[a] based on nominal content

Site characterization and product application details for the tea tree oil residue trial conducted on cucumbers in Germany are shown in Tables 9 and 10, respectively.

Table 9. Trial No. S08-02382-05 (1308) – Site Description

Location	
Postcode, town, county/federal state/département, country	Vetschau, Brandenburg, Germany 03226
Location reference/ GPS coordinates	51.81285N, 14.09731E
Crop	
Test system	Protected Cucumber (*Cucumis sativus*) - Bayer code ref. CUMSA .
Cultivar/Variety	Aramon
Planting or seeding date	11 Apr 2008
Plot Details	
Plot size width x length, other	2.5 m x 12 m
Closest distance between control and treated plot(s)	15 m
Closest distance between treated plots	4 m
Distance between rows	0.7m
plants per plot	44
TRV parameters	N/D
Slope	Flat

Table 10. Trial No. S08-02382-05 (1308) – Application Details

TREATMENT NO.	2	3
Application code	A1	A1
Application date	11 Sep 2008	11 Sep 2008
Application equipment	Knapsack sprayer	Knapsack sprayer
Nozzle type	Flat fan	Flat fan
Nozzle size	D5	D5
Spray pressure (bar)	8	8
Type of application	Directed application	Directed application
Application volume actual (L/ha)	1003	1008
g ai/hl applied*	119	238
L product /hl applied*	0.5	1.0
Crop growth stage (BBCH)	707	707
Stand height of crop (m)	2.3	2.3
Target conditions	Dry	Dry
Crop ground cover (%)	40	40
Actual air temperature (°C)	15.5	15.5
Wind speed (m/s)	na	na
Actual relative air humidity (%)	96.7	96.7
Cloud cover (%)	90	90
Wetness of soil - 2-5 cm	Dry	Dry
Temperature of soil - 2-5 cm (°C)	18.3	18.3

* based on nominal content

Site characterization and product application details for the tea tree oil residue trial conducted on cucumbers in the United Kingdom are shown in Tables 11 and 12, respectively.

Table 11. Trial No. S08-02382-06 (1309) – Site Description

Location	
Postcode, town, county/federal state/département, country	Cottingham, HU16 5RX, Yorkshire, UK
Location reference/ GPS coordinates	TA 041 339 53° 47' 29.23" N 00° 25' 13.74"
Crop	
Test system	Protected Cucumber (*Cucumis sativus*) - Bayer code ref. CUMSA
Cultivar/Variety	Aviance RZ
Planting or seeding date	14 Aug 2008
Plot Details	
Plot size width x length, other	3.2 m x 10 m
Closest distance between control and treated plot(s)	12 m
Closest distance between treated plots	10 m
Distance between rows	80cm
plants per plot	n/d
TRV parameters	n/d
Slope	Flat

Table 12. Trial No. S08-02382-06 (1309) – Application Details

TREATMENT NO.	2	3
Application code	A1	A1
Application date	24 Sep 2008	24 Sep 2008
Application equipment	Knapsack sprayer	Knapsack sprayer
Nozzle type	Lurmark LD08 F110	Lurmark LD08 F110
Nozzle size	nr	nr
Spray pressure (bar)	nr	nr
Type of application	Directed application	Directed application
Application volume actual (L/ha)	806	803
g ai/hl applied*	119	238
L product /hl applied*	0.5	1.0
Crop growth stage (BBCH)	89	89
Stand height of crop (m)	2.5	2.5
Target conditions	Dry	Dry
Crop ground cover (%)	80	80
Actual air temperature (°C)	22.0	22.0
Wind speed (m/s)	na	na
Actual relative air humidity (%)	92	92
Cloud cover (%)	95	95
Wetness of soil - 2-5 cm	Damp	Damp
Temperature of soil - 2-5 cm (°C)	19.5	19.5

* based on nominal content

Results

No residues above the limit of quantification were found in any of the untreated specimens.

Overall mean recovery of terpinen-4-ol, gamma-terpinene and 1,8-cineole in sub-samples of each crop matrix spiked at concentrations of 0.05 and 0.5 mg/kg were: 108% for terpinen-4-ol, 89% for gamma-terpinene and 94% for 1,8-cineole in tomatoes; 99% for terpinen-4-ol, 102% for gamma-terpinene and 109% for 1,8-cineole in sweet peppers; and 93% for terpinen-4-ol, 86% for gamma-terpinene and 89% for 1,8-cineole in cucumber fruits.

Analytical results for the six trials are shown in Tables 13-18. At all sites and for both test concentrations tea tree oil residues were below the limit of quantification of 0.05 mg/kg.

Table 13. BM 608 Residue Levels on Tomatoes Grown Indoors in Italy - Trial S08-02382-01 (1304)

Individual results, residues in mg/kg:

Sample No	Number and Nominal Rate of Application (g ai/hL)	Sampling Interval	Crop Part	Terpinen-4-ol residue (mg/kg)	Gamma-Terpinene residue (mg/kg)	1,8-cineole residue (mg/kg)
TREATED PLOT 2						
L08-02382-01-002A	1 x 119	0 HALA	Tomato fruits	<0.05	<0.05	<0.05
L08-02382-01-005A		6 HALA		<0.05	<0.05	<0.05
TREATED PLOT 3						
L08-02382-01-003A	1 x 238	0 HALA	Tomato fruits	<0.05	<0.05	<0.05
L08-02382-01-006A		6 HALA		<0.05	<0.05	<0.05
CONTROL PLOT U1						
L08-02382-01-001A	Control	0 HALA	Tomato fruits	<0.05	<0.05	<0.05
L08-02382-01-004A		6 HALA		<0.05	<0.05	<0.05

HALA: Hours After Last Application

No correction of results for either control residues or recovery values has been performed.

Table 14. BM 608 residue levels on sweet peppers grown indoors in Greece - Trial S08-02382-02 (1305)

Individual results, residues in mg/kg:

Sample No	Number and Nominal Rate of Application (g ai/hL)	Sampling Interval	Crop Part	Terpinen-4-ol residue (mg/kg)	Gamma-Terpinene residue (mg/kg)	1,8-Cineole residue (mg/kg)
TREATED PLOT 2						
L08-02382-02-002A	1 x 119	0 HALA	Sweet pepper fruits	<0.05	<0.05	<0.05
L08-02382-02-005A		6 HALA		<0.05	<0.05	<0.05
TREATED PLOT 3						
L08-02382-02-003A	1 x 238	0 HALA	Sweet pepper fruits	<0.05	<0.05	<0.05
L08-02382-02-006A		6 HALA		<0.05	<0.05	<0.05
CONTROL PLOT U1						
L08-02382-02-001A	Control	0 HALA	Sweet pepper fruits	<0.05	<0.05	<0.05
L08-02382-02-004A		6 HALA		<0.05	<0.05	<0.05

HALA: Hours After Last Application

No correction of results for either control residues or recovery values has been performed.

Table 15. BM 608 residue levels on cucumbers grown indoors in The Netherlands- Trial S08-02382-03 (1306)

Individual results, residues in mg/kg:

Sample No	Number and Nominal Rate of Application (g ai/hL)	Sampling Interval	Crop Part	Terpinen-4-ol residue (mg/kg)	Gamma-Terpinene residue (mg/kg)	1,8-Cineole residue (mg/kg)
TREATED PLOT 2						
L08-02382-03-002A	1 x 119	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-03-005A		6 HALA		<0.05	<0.05	<0.05
TREATED PLOT 3						
L08-02382-03-003A	1 x 238	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-03-006A		6 HALA		<0.05	<0.05	<0.05
CONTROL PLOT U1						
L08-02382-03-001A	Control	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-03-004A		6 HALA		<0.05	<0.05	<0.05

HALA: Hours After Last Application

No correction of results for either control residues or recovery values has been performed.

Table 16. BM 608 residue levels on cucumbers grown indoors in France- Trial S08-02382-04 (1307)

Individual results, residues in mg/kg:

Sample No	Number and Nominal Rate of Application (g ai/hL)	Sampling Interval	Crop Part	Terpinen-4-ol residue (mg/kg)	Gamma-Terpinene residue (mg/kg)	1,8-Cineole residue (mg/kg)
TREATED PLOT 2						
L08-02382-04-002A	1 x 119	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-04-005A		6 HALA		<0.05	<0.05	<0.05
L08-02382-04-008A		12 HALA		<0.05	<0.05	<0.05
TREATED PLOT 3						
L08-02382-04-003A	1 x 238	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-04-006A		6 HALA		<0.05	<0.05	<0.05
L08-02382-04-009A		12 HALA		<0.05	<0.05	<0.05
CONTROL PLOT U1						
L08-02382-04-001A	Control	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-04-004A		6 HALA		<0.05	<0.05	<0.05
L08-02382-04-007A		12 HALA		<0.05	<0.05	<0.05

HALA: Hours After Last Application

No correction of results for either control residues or recovery values has been performed.

Table 17. BM 608 residue levels on cucumbers grown indoors in Germany - Trial S08-02382-05 (1308)

Individual results, residues in mg/kg:

Sample No	Number and Nominal Rate of Application (g ai/hL)	Sampling Interval	Crop Part	Terpinen-4-ol residue (mg/kg)	Gamma-Terpinene residue (mg/kg)	1,8-Cineole residue (mg/kg)
TREATED PLOT 2						
L08-02382-05-002A	1 x 119	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-05-005A		6 HALA		<0.05	<0.05	<0.05
L08-02382-05-008A		12 HALA		<0.05	<0.05	<0.05
TREATED PLOT 3						
L08-02382-05-003A	1 x 238	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-05-006A		6 HALA		<0.05	<0.05	<0.05
L08-02382-05-009A		12 HALA		<0.05	<0.05	<0.05
CONTROL PLOT U1						
L08-02382-05-001A	Control	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-05-004A		6 HALA		<0.05	<0.05	<0.05
L08-02382-05-007A		12 HALA		<0.05	<0.05	<0.05

HALA: Hours After Last Application

No correction of results for either control residues or recovery values has been performed.

Table 18. BM 608 residue levels on cucumbers grown indoors in the UK - Trial S08-02382-06 (1309)

Individual results, residues in mg/kg:

Sample No	Number and Nominal Rate of Application (g ai/hL)	Sampling Interval	Crop Part	Terpinen-4-ol residue (mg/kg)	Gamma-Terpinene residue (mg/kg)	1,8-Cineole residue (mg/kg)
TREATED PLOT 2						
L08-02382-06-002A	1 x 119	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-06-005A		6 HALA		<0.05	<0.05	<0.05
L08-02382-06-008A		24 HALA		<0.05	<0.05	<0.05
L08-02382-06-011A		48 HALA		<0.05	<0.05	<0.05
TREATED PLOT 3						
L08-02382-06-003A	1 x 238	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-06-006A		6 HALA		<0.05	<0.05	<0.05
L08-02382-06-009A		24 HALA		<0.05	<0.05	<0.05
L08-02382-06-012A		48 HALA		<0.05	<0.05	<0.05
CONTROL PLOT U1						
L08-02382-06-001A	Control	0 HALA	Cucumber fruits	<0.05	<0.05	<0.05
L08-02382-06-004A		6 HALA		<0.05	<0.05	<0.05
L08-02382-06-007A		24 HALA		<0.05	<0.05	<0.05
L08-02382-06-010A		48 HALA		<0.05	<0.05	<0.05

HALA: Hours After Last Application

No correction of results for either control residues or recovery values has been performed.

Study Author's Conclusions

No specific conclusions given.

Reviewer's Conclusion

Acceptable. Although it might be argued that to support a statistical analysis, multiple samples should have been analyzed at each time point (instead of only one), the consistency of the results across time point and trial sites support the conclusion that multiple analyses would not have changed the results.

DATA EVALUATION RECORD

Tea Tree Oil

STUDY TYPE: Ready Biodegradability - CO_2 in Sealed Vessels (Headspace Test) (OCSPP 835.3120)

MRID 48275004
[Includes Amendment]

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Summitec Corporation
9724 Kingston Pike, Suite 602
Knoxville, Tennessee 37922

Task 4, Work Order No. 11-024

Primary Reviewer:
Dennis M. Opresko, Ph.D.

Signature: _____
Date: JAN 0 4 2012

Secondary Reviewers:
Anthony Armstrong, Ph.D.

Signature: Anthony Q. Armstrong, AE
Date: JAN 0 4 2012

Robert H. Ross, M.S. Program Manager

Signature: _____
Date: JAN 0 4 2012

Quality Assurance:
Angie Edmonds, B.S.

Signature: Angie Edmonds
Date: JAN 0 4 2012

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Date: _____

Robert H. Ross, M.S. Program Manager Signature: _____
Date: _____

Quality Assurance:
Angie Edmonds, B.S. Signature: _____
Date: _____

Disclaimer

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DATA EVALUATION RECORD

EPA Secondary Reviewer:	**Angela L. Gonzales /s/ 1/24/13**
STUDY TYPE:	Biodegradation (835.3120)
MRID NO:	48275004
DP BARCODE:	396175
DECISION NO:	408891
SUBMISSION NO:	903519
TEST MATERIAL:	Tea Tree Oil
STUDY NO:	AHT13279
SPONSOR:	Biomor Israel Ltd., PO Box 81, Qatzrin, 12900 Israel
TESTING FACILITY:	Dr. U.Noack-Laboratorien, Kathe-Paulus Strabe 1, D-31157 Sarstedt, Germany
TITLE OF REPORT:	Tea Tree Oil Ready Biodegradability CO_2 in Sealed Vessels (Headspace Test) [includes Amendment]
AUTHOR:	S. Fiebig
STUDY COMPLETED:	July 15, 2010
CONFIDENTIALITY CLAIMS:	No claim of confidentiality
GOOD LABORATORY PRACTICE:	"This study does not meet the requirements of 40 CFR Part 160. This study was conducted in accordance with the Good Laboratory Practice Regulations of the OECD [OECD series number 1, ENV/MC/CHEM (98) 17, 1998]."
STUDY SUMMARY:	A 28-day ready biodegradability assay (CO_2 in sealed vessels; Headspace Test) was conducted on tea tree oil (batch KY-4) according to OECD Guideline 310 and EN ISO 14593 (1999). The test concentration was 10.2 mg C/L. Biodegradation was determined by TIC analysis of the amount of CO_2 produced over time. Results for test and reference substance were corrected for endogenous TIC production in the control groups and the blind value of a sodium hydroxide solution. A functional control was used to check the activity of the system, and a toxicity control (test and reference substance combined) was used to check for inhibition. Biodegradation of the test substance was 10% after 2 days, and the 60% pass level was reached within the 10-day window after 5 days. Maximum biodegradation came to 106% after 28 days (95% CI = 104-109%).
CLASSIFICATION:	**Acceptable.**

Test Material

Tea Tree Oil (Batch No. KY-4): 10.433% w/w α-terpinene; 3.277% w/w 1,8-cineole; 21.667% w/w g-terpinene; 2.277% w/w p-cymene; 40.067% w/w terpinene-4-ol, totaling 77.7%. A Certificate of Analysis was included in the study report (p. 23 of MRID 48275004). The test material was stored at room temperature and protected from moisture and light.

Background

In October 6, 2010, the registrant submitted biodegradation data in support of the pending registration of Tea Tree Oil Technical (EPA Reg. No. 86182-E; 100% A.I.) and the end use product Timorex Gold (EPA Reg. No. 86182-R; 23.8% A.I.). The registrant stated that the submitted data were not to address any deficiencies in the submission, but to help create a complete data set on the active ingredient and proposed end use product.

Methods and Materials

Test Method: OECD Guideline 310 and EN ISO 14593 (1999); Headspace Test (1:2 headspace to liquid ratio) in which ready biodegradation is determined over a period of 28 days with a non-adapted activated sludge by monitoring the release of CO_2. Determination of CO_2 was carried out by IC analysis with a carbon analyzer according to DIN EN 1484. The tests included the test substance at 10.2 mg C/L; a functional control [100% sodium benzoate, at a concentration of 30 mg/L (17.5 mgC/L)]; a toxicity control (test substance plus 20 mg/L of the reference item, sodium benzoate); and an inoculum control (inoculum without test substance or reference substance). For each sampling date there were three replicates of each test; at test end there were five replicates of each. Analyses were made at the start of the test, twice in the first two weeks, and thereafter every 7 days.

The amount of TIC produced was calculated by correcting the results of the test item and reference item for endogenous TIC production of the control groups and the blind value of the sodium hydroxide solution.

The biodegradation was calculated from the ratio theoretical TIC concentration at test start (ThIC = TOC) to net TIC production.

TIC = (mg C in the liquid + mg C in the headspace)
= (VL x CL) + (VH x CH)

VL = volume of liquid in the bottle (litre)
CL = concentration of IC in the liquid (mg/L as carbon)
VH = volume of the headspace (litre)
CH = concentration of IC in the headspace (mg/L as carbon)

$$\%D = \frac{(TIC_t - TIC_b)}{TOC} \times 100$$

%D = biodegradation (%)
TIC_t = mg TIC produced in test bottle at time t
TIC_b = mean mg TIC produced in control bottles at time t
TOC = mg TOC added initially to the test vessel

The mean amount of total inorganic carbon (TIC) produced in the inoculum controls at the end of the test should be < 15 % of the organic carbon added initially as the test item.

The 95 % confidence interval for the mean percentage biodegradation after 28 days was calculated using software SigmaPlot.

Inoculum: A non-adapted activated sludge from the sewage treatment plant at Hildesheim, Germany was used as the inoculum. The plant receives predominantly municipal sewage and "hardly" any industrial chemical waste.

Validation Criteria: The percentage degradation of the reference item had to exceed the pass level of 60% by day 14. The mean amount of TIC present in the inoculum control at the end of the test had to be less than 3 mg C/L.

Results

The Validation Criteria were met; degradation of the functional control reached 60% by day 5 and the mean amount of TIC in the inoculum at test end was 1.66 mg C/L.

An amendment dated 19.7.10 was added to the report because the confidence intervals for the test item, functional control, and toxicity control on day 28 had to be revised.

Results of the assay, as they were presented in the amendment to the report, are shown in Table 1. Results for the Toxicity Control indicated that degradation of the reference item was not inhibited by the test substance. Biodegradation of the test substance was 10% after 2 days and the 60% pass

level was reached within the 10-day window after 5 days (Table 1). Mean percent degradation of the test item was 87% by day 7 and 106% by day 28 (Table 2).

Table 1. CO_2 Production and Biodegradation of Tea Tree Oil.

	CO_2-Production in mg C/L							
Day	P_1	P_2	P_3	P_4	P_5	MP	Net	Degr. [%]
0	2.16	1.88	2.13			0.00	–	–
1	2.55	2.37	2.72			0.84	0.00	0
5	10.20	10.36	10.47			8.80	7.57	74
7	12.90	13.11	12.86			10.80	8.90	87
9	12.90	12.62	12.43			10.22	8.41	82
14	13.04	13.05	13.19			10.87	9.20	90
21	15.40#	15.38#	15.90#			13.72	11.02	108
28	14.82	14.50	14.99	14.82	14.67	12.51	10.85	106

MP = mean production (NaOH corrected)
Degr. = degradation
# = measured after storing in refrigerator for 8 days at 6 ± 2 °C

Table 2. Biodegradation and Confidence Interval for Tea Tree Oil in Comparison to the Functional Control and Toxicity Control.

	Biodegradation [%] / Day [d]				Confidence Interval on Day 28
	7	14	21	28	P = 95 %
Test Item, (Tea Tree Oil, 10.2 mg C/L)	87	90	108	106	104 - 108
Functional Control (Sodium benzoate, 30 mg/L)	75	79	94	89	86 - 92
Toxicity Control 10.2 mg C/L Test Item (Tea Tree Oil) + 20 mg/L Reference Item (Sodium benzoate)	78	83	86	87	85 - 89

Study Author's Conclusions

The test item must be regarded as readily biodegradable in the 10-day window, and after 28 days.

Reviewer's Conclusion

Acceptable.

NOTE: The test material in this biodegradation study was Tea Tree Oil, Batch KY-4, for which the percent composition of five of the active ingredients were identified (see Test Material section). The CSF (dated 10/6/10) for end product Timorex Gold indicates that it contains 23.8% Tea Tree Oil Technical (100% purity), with the remaining 76.2% of the product being solvents, emulsifier, surfactant and neutralizer. The impact of the other ingredients in Timorex Gold on its biodegradability cannot be ascertained from the results of the test on Tea Tree Oil.

REFERENCES

(1) **OECD-Guideline 310 for Testing of Chemicals, Ready Biodegradability – CO_2 in Sealed Vessels (Headspace Test) (March 2006)**

(2) **EN ISO 14593, Wasserbeschaffenheit – Bestimmung der vollständigen biologischen Abbaubarkeit organischer Substanz im wässrigen Medium – Verfahren mittels Bestimmung des anorganischen Kohlenstoffs in geschlossenen Flaschen (CO_2-Headspace-Test) (1999)**

(3) **DIN EN 1484, Wasseranalytik – Anleitungen zur Bestimmung des gesamten organischen Kohlenstoffs (TOC) und des gelösten organischen Kohlenstoffs (DOC)**